Effect of plantain (*Plantago lanceolata* L.) proportion in the diet on nitrogen use, milk production and behaviour of lactating dairy cows

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Daniel Nkomboni

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Expansion and intensification of dairy in pasture based systems whilst economic, creates environmental challenges through nitrate leaching and degradation of water quality. With pressure to reduce nitrate leaching and simultaneously increase pasture productivity and milk production, studies have been carried out to develop management strategies to reduce N surpluses. At the animal level an effective way to reduce the environmental impacts is through nutritional interventions. Inclusion of plantain (*Plantago lanceolata* L.), in the traditional perennial ryegrass-white clover pastures has shown potential to reduce nitrogen concentration in urine. Most studies including plantain however, grew it in diverse pastures where it is difficult to manage persistence due to different grazing requirements compared with perennial ryegrass. Further its contribution to N loss when sown in a mix with other plant species was not clear.

This research evaluated the effect of grazing increasing proportions of spatially planted plantain and perennial ryegrass-white clover pastures species on N excretion, milk production, urinary and feeding behaviour in Canterbury, New Zealand. The experiment was carried out at Lincoln University Research Dairy Farm, Canterbury, New Zealand in April 2016. Forty eight late lactating primi and multi-parous Friesian x Jersey cows were blocked according to pre-experimental average (mean, ± s.e.m) liveweight (499.21 ± 6.44 kg), milk solids (1.41 ± 0.02 kg), milk yield (14.14 ± 0.29 kg) and age (5.42 ± 0.19). Four cows were randomly allocated to 3 replicates of 4 treatments (0% plantain, 15% plantain, 30% plantain, 60% plantain) of proportions of spatially separated perennial ryegrass-white clover and pure plantain.

Pre-grazing herbage mass (kg/ha DM ± sem) for pasture and plantain were similar across treatments (3857.9 ± 46.5 and 4973.1 ± 74.5 respectively) whilst the cows grazed the two monocultures to the same post-grazing herbage mass (1504.8 ± 36.1 *vs*. 1510.5 ± 79.3). Chemical composition was similar between perennial ryegrass-white clover and plantain across treatments. Pre-grazing pasture had similar CP and ME (23.6 ± 0.61%, 12.3 ± 0.03 ME/kg DM and 20.5 ± 0.24% and 12.77 ± 0.04 ME/kg DM for perennial ryegrass-white clover and plantain respectively. Dry matter intake (DMI) estimated from pre- and post-grazing herbage mass was lower (P=0.003) for PL0 than PL15, PL30 and PL60 and similar between PL15, PL30 and PL60 (14.74 vs. 15.96, 16.00, 15.97kg respectively). Milk yield (16.1 L/cow/day), milk solids (1.6 L/cow/day), protein (4.5%) and fat (5.8%) were unaffected by percent proportion increase of plantain in the diet. Urine N concentration and urine N output had 33% lower N (P=0.012) in the PL60 than PL0 (0.30 *vs*. 0.45 g N/L and 431.3 g N /day *vs*. 545.4 g N/day respectively). The effect was mainly related to the difference derived from 30% to 60% plantain in the diet with the difference in N concentration and N output between PL0 and PL30 being 13%. Milk, urea and milk urea N declined (P=0.004) by 18% in PL60 compared with PL0. The urine volume (2.3 litres/urination) and patch area (0.34m^2) were unaffected by the percent increase of plantain proportions in the diet. Based on urine volume, patch size and N concentration, there was a 40% decline from 316 to 190 kg N/ha in urine patch loading from 0% to 60% proportion of plantain in the diet indicating the key potential of plantain to reduce N loading in pastures. As urine loading is critical for determining N leaching, this is likely to contribute to reduced leaching even if urine frequency is marginally higher. The cows allocated more time (47.3 minutes) to plantain than pasture (43.54 minutes) during the morning grazing, but in the afternoon grazing bout, the reverse was true with more time spent grazing pasture than plantain (62.1 vs. 34.9 minutes/cow respectively). Animal bite rates were similar in the morning and afternoon grazing bouts (46 *vs*. 39 and 49 *vs*. 44 bites/minute/cow respectively).

This study confirmed that plantain has similar feed value and milk production potential to perennial ryegrass-white clover and when offered as green leafy herbage to dairy cows. However, feeding greater than 30% plantain in the diet resulted in significant reduction in both urine N concentration and urine N excretion. As these are key components of determining N loading of urine patches, the study indicates that plantain may present appealing opportunities to reduce environmental impact of dairy farming.

Keywords: plantain, ryegrass-white clover, nitrogen, proportions, urinary nitrogen

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YOUR WILL BE DONE.

Table of Contents

List of Tables

List of Figures

List of abbreviations

ANOVA	Analysis of variance
ADF	Acid detergent fibre
BCS	Body condition score
BUN	Blood urea nitrogen
CP	Crude protein
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DOMD	Digestibility of organic matter in dry matter
DW	Dry weight
FCE	Feed conversion efficiency
LWT	Liveweight
masl	metres above sea level
ME	Metabolisable energy
MJ ME/kg DM	Mega joules of metabolisable energy / kg of dry matter
MS	Milk solids
MUN	Milk urea nitrogen
N	Nitrogen
NDF	Neutral detergent fibre
NH_3	Ammonia
NIRS	Near infrared reflectance spectroscopy

OM	Organic matter
PD	Purine derivatives
pH	Potential of hydrogen
RPM	Rising plate meter
WSC	Water soluble carbohydrates
SEM	Standard error of mean

Chapter 1

Introduction

1.1 Background

Pasture based systems are an important part of maintaining New Zealand dairy farming industry's international competitiveness (Pembleton, Tozer, Edwards, Jacobs, & Turner, 2015). These systems have been traditionally based on a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) because this pasture persist longer than other pastures under seasonal temperature and moisture changes, produce high dry matter (12-15 t DM/ha/year) and can withstand intensive grazing by sheep and cattle and (DM)/ha (Langer, 1990; Moot, Matthew, & Kemp, 2007). Pasture based systems are driven by summer rainfall, irrigation and the use of nitrogen fertiliser. Traditional ryegrass-white clover pastures are characterised by low efficiency of feed N utilisation leading to excretion of over 50% dietary N in the urine (Bhat, Kannan, Singh, & Sharma, 2013; Bryant, Gregorini, & Edwards, 2012; Carulla, Kreuzer, Machmüller, & Hess, 2005). Expansion and intensification of pasture based dairying whilst economic, is restricted by its environmental footprint mainly through nitrate leakage to ground water sources and waterways (Beukes et al., 2012; Bryant, Gregorini, *et al.*, 2012). The growing pressure to reduce N leakage (predominantly nitrate leaching) and the need to increase the productivity of pastures and milk (Woodward, Waugh, Roach, Fynn, & Phillips, 2013), have led to research on management strategies to reduce N surpluses at the landscape and animal level (Beukes *et al.*, 2012; Monaghan *et al.*, 2007). Some of the nutritional strategies, involving diverse pastures of ryegrass-white clover or ryegrass and herbs (plantain and chicory) suggest the potential to mitigate N environmental challenges created by intensification. Compared with other herbs, plantain (*Plantago lanceolota* L.) has not been extensively evaluated for its contribution to DM production in diverse pastures (Powell, Kemp, Jaya, & Osborne, 2007).

Plantain is an erect-growing temperate and subtropical palatable mineral rich perennial herb that tolerates many ecological environments including natural grasslands, road sides and cropped lands (Grigore, Bubueanu, Pirvu, Ionita, & Toba, 2015). It originates in Eurasia (Webb, Sykes, & Garnock-Jones, 1988) with countries that include south and east Iceland, Spain, northern and central Asia being mentioned in its history (Grigore *et al.*, 2015).

Plantain has been selected and bred in New Zealand to improve dry matter (DM) production and persistence for livestock farm systems (Stewart, 1996). Because plantain tolerates dry (summer) and cold (winter and early spring) seasons, it produces more dry matter than chicory (17 t DM/ha *vs*. 14 t

DM/ha) (Powell *et al.*, 2007). Comparing a ryegrass-white clover and plantain mix with ryegrass-white clover on farms in Northland over 3 years, Moorhead and Piggot (2009), recorded an average yearly DM production of 17.6 t DM/ha for plantain based pastures compared with 14.3 t DM/ha for ryegrass-white clover pastures. The dominance of plantain based pasture in that study was exhibited in summer and autumn when the herbs produced significantly more DM than ryegrass-white clover pastures by 1.8 t DM/ha and 0.9 t DM/ha respectively.

Plantain also contains a range of active bio-chemical compounds that may affect animal health and production (Rumball, Keogh, Lane, Miller, & Claydon, 1997). Its antimicrobial properties interact with fermentation to reduce gas production and bloat in ruminants (Navarrete, Kemp, Pain, & Back, 2016). It also contains starch that promotes propionic producing bacteria increasing milk yields and has mild anthelmintic effects on intestinal parasites (Deaker, Young, Fraser, & Rowarth, 1994). Plantain is also used in commercial products that control diarrhoea in calves as it contains 0.8% mucilage (Brautigam & Franz, 1985; Duke, 1992). The herb has 0.4 -1% condensed tannins that could potentially change the site of digestion of proteins from the rumen to the intestine thus increasing the efficiency of N utilisation (Terrill, Rowan, Douglas, & Barry, 1992). Plantain also contains higher macro (P, K, Ca, Mg, Na) and micro (Bo, Cu, Mn, Zn) nutrient concentration (Sanderson, Labreveux, Hall, & Elwinger, 2003) than pasture. Finally plantain contains iridoid glycosides that may have diuretic tendencies (Grigore *et al.*, 2015). Green pastures are known to contain higher levels (>5mg/kg DM) of K that increases dietary cation-anion difference (DCAD) in lactating cows leading to hypocalcaemia (Sanderson *et al*., 2003). Rugoho, Gourley, and Hannah (2016) reported reduced DCAD for plantain and rape mix compared with ryegrass pasture (171 *vs*. 258 mEq/kg DM respectively).

Totty, Greenwood, Bryant, and Edwards (2013), reported reduced N urinary excretion when diverse pastures (plantain, chicory, lotus and high sugar ryegrass-white clover) were compared with ryegrass-white clover (353.8 g/day *vs*. 426.6 g/day respectively) for late lactation cows. In this trial, it was postulated that the partitioning of N to milk production was due to the condensed tannins in the herbs. Urinary N concentration of late lactating cows grazing perennial ryegrass-white clover pastures and 50:50 plantain-ryegrass in autumn, was 33% lower (5.4g N/L *vs*. 3.6g N/L) (Box, Edwards, & Bryant, 2016). Consistent with this publication, Edwards *et al*. (2015), found urinary N concentration 20% lower for cows grazing diverse (4.9g N/L) than those on ryegrass-white pastures (6.1g N/L).

Cheng *et al*. (2015) measured urinary excretion on heifers grazing perennial ryegrass-white clover (PA), chicory (CH), PA +CH, plantain (PL) and PL + PA and reported no significant difference in urinary nitrogen excretion per heifer amongst the treatments (67.0, 74.2, 69.8, 72.4 and 79.9 g N/day respectively). However Cheng *et al*. (2015) did find higher urination frequency of heifers consuming chicory and plantain + chicory (during the day) than ryegrass-white clover or plantain and attributed the high urination frequency to diuretic effects. There are different diuretic forms and because of the high mineral content in chicory (potassium, calcium, sodium) an osmotic diuresis was suspected. However this did not explain why those grazing plantain also of high mineral content (calcium, magnesium, sodium, phosphorus, zinc) (Stewart, 1996) had lower urination frequencies. In sheep grazing plantain or ryegrass-white clover, O'Connell, Judson, and Barrell (2016) produced evidence that plantain has a water diuretic effect. Sheep on plantain produced higher urine volume by 1.7L on the first day of the trial than those on ryegrass-white clover and 0.5L more on 5 subsequent collection days.

1.1.1 Problem statement

The challenges currently affecting the New Zealand dairy industry are the negative impacts of increased N leakage on the environment and seasonality of pasture DM supply on milk solids production (Rawnsley *et al*., 2013). Dietary nitrogen (N) intake is the main cause of N excretion to the environment from the traditional ryegrass-white clover pastures. This is because these pastures contain 3.2-3.9% N in DM (Ledgard, Sprosen, Penno, & Rajendram, 2001) which exceeds animal requirements of 3% N in DM (Pacheco & Waghorn, 2008) for lactating dairy cows.

Temperate pasture based systems typically have low N utilisation efficiency leading to low efficiency N escaping in urine increasing the N footprint on the environment (Bhat *et al*., 2013; Bryant, Gregorini, *et al*., 2012; Carulla *et al*., 2005).

Research on the complementarities of alternative herbs with traditional pastures and their effects on animal performance is still inconsistent and inconclusive. This is because there is variation in scientific evidence on integration of pastures to improve animal production and reduce environmental pollution. Past research (Box *et al*., 2016; Edwards *et al*., 2015; Judson & Edwards, 2016; Nobilly, Bryant, McKenzie, & Edwards, 2013; Totty *et al.*, 2013) has indicated that plantain is important for reducing N excretion but it is not clear what quantities of it are required in the diet. In *situ* grazing of plantain at different proportions with perennial ryegrass-white clover in spatial patterns in dairy may therefore be necessary.

1.1.2 Justification

Plantain and other herbs have been incorporated into pastures to form diverse pastures resulting in increased DM production and reduction of N excretion (Nobilly *et al*., 2013; Totty *et al*., 2013; Woodward *et al*., 2013). While there is evidence supporting the positive effects of plantain on urinary N loss, there is no information on how much plantain is required to have an impact on N loss.

Most studies on diverse pastures have included plantain in the mix. As nutrient management becomes increasingly regulated and monitored, the effect of one plant species in a mixed pasture will be difficult to estimate due to fluctuations in botanical composition caused by season and management. Data is required to determine what proportion of the diet plantain should consist of to support high milk yield and reduce urinary N loss.

1.1.3 Objectives

This research aimed to evaluate the effect of grazing spatially planted plantain and perennial ryegrass-white clover pastures on urinary N excretion, milk production and urinary and feeding behaviour of late lactating dairy cows grazing irrigated pastures in Canterbury, New Zealand in autumn.

The objective of the research was to examine the effect of feeding different dietary proportions of plantain (0%; 15%; 30%; 60%) on milk production, milk composition, urinary N excretion and composition, faecal N concentration and grazing behaviour of late lactation dairy cows in autumn.

1.1.4 Hypothesis

Proportions of pure plantain with perennial ryegrass-white clover alter nitrogen partitioning, milk production and grazing and urination behaviour of late lactating dairy cows.

1.1.5 Thesis outline

This thesis is composed of 5 chapters. Chapter 1 highlights the background of the project citing relevant literature to support it. The chapter briefly states the problem and discusses the justification for doing the research. Furthermore, the objectives, research questions and the main hypothesis are outlined. Chapter 2 reviews the literature associated with the effect of forages notably plantain on milk production and nitrogen excretion. Literature review topics include: plantain origins, growth characteristics and its biochemical compounds, effects of plantain on N excretion, plantain DM production, nutritive value and relationship with milk production, N excretion and behaviour (grazing and urination). This chapter compares plantain with ryegrass-white clover pastures on these parameters. Chapter 3 describes the materials and methods used to collect and process data. Chapter 4 presents the results of the measured parameters following both laboratory and statistical analysis. Herbage characteristics, animal parameters (milk, plasma, urine and faeces), grazing and urination behaviour results are tabled. Chapter 5 discusses the results with aid of supporting literature. This chapter draws conclusions and suggests recommendations for use by farmers and further research. Chapter 6 concludes the findings by bringing multiple aspects coherently together in a discussion.

Chapter 2 Literature review

2.1 Introduction

Expansion and intensification of the dairy in New Zealand whilst economic, is challenged by its environmental footprint mainly through nitrate (NO_3^-) leakage to ground water sources and waterways (Beukes *et al*., 2012; Bryant, Gregorini, *et al*., 2012). In the temperate pasture based systems with high soluble rumen degradable protein, N escapes in urine due to low efficiency of utilisation in the cows' rumen (Bhat *et al*., 2013; Bryant, Gregorini, *et al*., 2012; Carulla *et al*., 2005). The notion that a farm's success can be measured by production of DM to support milk production no longer holds as other factors like droughts and environmental regulations that include control of water and nitrogen fertilisers are important (Woodward *et al*., 2013). With growing pressure to reduce the nitrogen leakage while increasing pasture productivity and milk production, studies have been carried out to develop management strategies to reduce N surpluses at landscape and animal level (Beukes *et al*., 2012; Monaghan *et al*., 2007). Inclusion of plantain and other herbs in the traditional ryegrass-white clover pastures or ryegrass pastures has been researched mainly in sheep (Judson, McAnulty, & Sedcole, 2009a; Raeside, Nie, Robertson, Partington, & Behrendt, 2014) with limited work done on dairy systems that contribute more N to the environmental foot print (Monaghan, de Klein, & Muirhead, 2008).

New Zealand pasture based systems are associated with seasonality of forage supply and nutritive value (Rawnsley *et al*., 2013). Peak DM production of pastures is reached in spring, followed by slower growth with increasing temperatures and soil moisture deficits in summer and autumn. There is a slight increase in DM with the autumn rains and falling temperatures and finally growth reduction with winter temperatures and water logging (Pembleton *et al*., 2015). With this in mind integration of pasture species with varying seasonal growth patterns will even out forage supply and present pasture combinations that achieve high DM and nutritive value (Jacobs & McKenzie, 2003). Plantain can supplement DM when perennial ryegrass-white clover pastures have low DM in summer and autumn. Moorhead and Piggot (2009) reported that plantain produced 1.8 t DM/ha and 0.9 t DM/ha more than ryegrass-white clover pastures in summer and autumn respectively. Plantain is also tolerant to low winter and early spring temperatures compared with ryegrass-white clover pastures and therefore could be used to increase DM when other pastures re-growth is slow.

This literature review assesses how spatial integration of plantain with perennial ryegrass-white clover can influence dairy animal performance and reduce environmental impact. The aim is to develop plantain monoculture proportions in perennial ryegrass-white clover-based pastures for increased performance in lactating primi and multi-parous dairy cow performance and reduced nitrogen impact on the environment.

2.2 Plantain origins and growth characteristics

Plantain (*Plantago lanceolata* L.) also known as ribwort plantain belongs to the *Plantaginaceae* family (Grigore *et al*., 2015). It is an erect perennial palatable mineral rich forage herb of the temperate regions. The herb tolerates summer temperatures and therefore is also distributed across subtropical regions (Stewart, 1996). Plantain is native to Eurasia (Webb *et al*., 1988) with south and east Iceland, Spain, northern and central Asia being mentioned as sites of origin (Grigore *et al*., 2015). Grasslands Lancelot and Ceres Tonic pasture plantain cultivars were developed in New Zealand from local strains of European genetic material (Stewart, 1996). These two pasture cultivars differ in growth and seasonality and fit well with seasonal variation in New Zealand (Table 2.1).

Generally perennial plantain herbs have an erect growth habit. Of the two cultivars developed in New Zealand (Table 2.1), Grasslands Lancelot is bushy, less erect and more suitable to summer growth whilst Ceres Tonic is very erect and tolerates both winter and summer seasons (Stewart, 1996). The characteristics of plantain (Table 2.1) explain why it tolerates drought, winter temperatures and common diseases and pests better than ryegrass and white clover (Stewart, 1996). The common flatweed type is less robust and prostrate in growth and hence produces less DM as compared to the other two cultivars.

Table 2.1 Common cultivated plantain varieties in New Zealand (Source: Stewart (1996))

	Grasslands Lancelot	Ceres Tonic	Common flatweed type
Growth habit	Semi-erect, bushy	Very erect	Prostate
Leaf size	Medium to large	Very large	Small to medium
Tiller numbers	High	Medium	Medium to high
Winter growth	Low	High	Very low
Summer growth	High	High	Low

2.3 Dry matter production

Dry matter production (DM) and nutritional characteristics of the pasture are the main drivers to animal performance and strategies that promote these drivers advance livestock production (Raeside *et al*., 2014). Limited information has been published on the growth and development of perennial forage species, especially plantain (Powell *et al*., 2007). Understanding plantain growth and development over time is essential for developing grazing management strategies in dairy systems.

2.3.1 Leaf and root growth

Thermal time affected the growth of leaves of plantain with the herb producing 6 leaves at 600 ^{o}C.day and declining at higher thermal time thereafter. Plantain tended to allocate less DM to roots than to shoots with the root to shoot dry weight decreasing exponentially with the increase in thermal time. (Powell *et al*., 2007). Sanderson and Elwinger (2000) made the same observations regarding allocation of DM to roots, but also reported that plantain produced 8-10 adventitious roots.. The authors also observed that in autumn leaf growth was slower (2 leaves) than in spring with leaf development of 6-7 leaves in 40 to 50 days after planting for plantain an observation also made by Powell *et al*. (2007). Sanderson and Elwinger (2000) concluded that 3- 4 leaves for plantain is necessary for successful establishment of seedlings whilst Powell *et al*. (2007) noticed that delaying grazing to 765 ^{o}C.day results in less leaf loses (10%) and persistence of plantain. At 765^{o}C.day the leaves will have developed to about 6 and plantain can be grazed when they reach 30 cm (Powell *et al*., 2007).

2.3.2 Dry matter production of plantain monocultures

It has been shown that pastures containing plantain grow in summer when productivity of perennial ryegrass-white clover constrains livestock production. When the growth and development of plantain (Ceres Tonic) was evaluated for their DM production at 8, 12 and 19 weeks after sowing, plantain produced high DM. (Powell *et al*., 2007). Plantain produced 17 t DM. Herbage accumulation (kg DM/ha) at 8, 12 and 19 weeks followed an exponential growth curve increasing from 8 to 19 weeks. (Table 2.2). Accumulated DM production rate (kg DM/ha/day) indicated the highest DM production during the spring period (Figure 2.1). Grazing 8 weeks after sowing resulted in >30% plant losses with fewer losses (3%) at 19 weeks grazing after sowing.

Table 2.2 Dry matter production (kg/ha) of plantain just before first grazing times at 8, 12 and 19 weeks after sowing (Source: Powell *et al*. (2007))

	Dry matter production (kg/ha)		
Species	8 weeks	12 weeks	19 weeks
Plantain	849	2606	5216

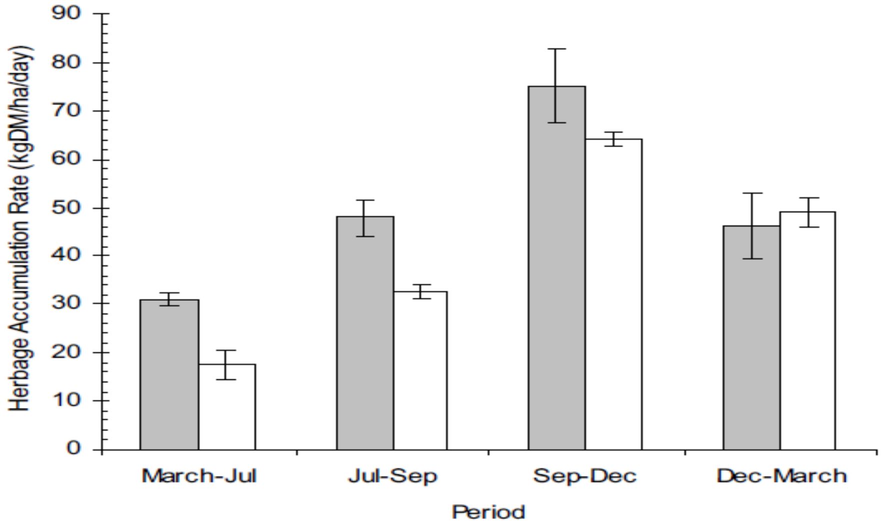

Figure 2.1 Herbage accumulation rate of plantain (grey) and chicory (white) for periods from March 2006 to March 2007. Bars show standard error of means (Source: Powell *et al*. (2007))

2.3.3 Diverse pastures dry matter production

Plantain grazing in monocultures may be limited as it cannot withstand heavy grazing like perennial ryegrass (Moorhead & Piggot, 2009). Kemp, Kenyon, and Morris (2010) suggested that under New Zealand rotational grazing systems for sheep and cattle, herbs (chicory and plantain) can persist for 2- 5 years whilst perennial ryegrass-white clover pastures can grow up to 10 years although their productivity declines after 3-5years.

The reports of the effect of herbs (plantain and chicory) on perennial ryegrass based pastures on DM and milk production are inconsistent. The effect of adding Ceres Tonic to ryegrass-white clover pastures was evaluated on farms in Northland over 3 years (Moorhead & Piggot, 2009). Average yearly DM production was 17.6 t DM/ha for plantain based-pastures compared with 14.3 t DM/ha for ryegrass-white clover pasture (Figure 2a). The advantage of the plantain based pastures over the ryegrass-white clover pastures was 6 t DM/ha in the first year and 1.2 t DM/ha by year 3 and this represented a 32 to 90% greater yield over 3 years. The dominance of plantain based pasture was exhibited in summer and autumn when they produced significantly more than ryegrass-white clover pastures at 1.8 t DM/ha and 0.9 t DM/ha respectively (Figure 2.1b). The DM production was similar in other seasons indicating that plantain tolerates dry conditions and higher temperatures than ryegrass and white clover pastures.

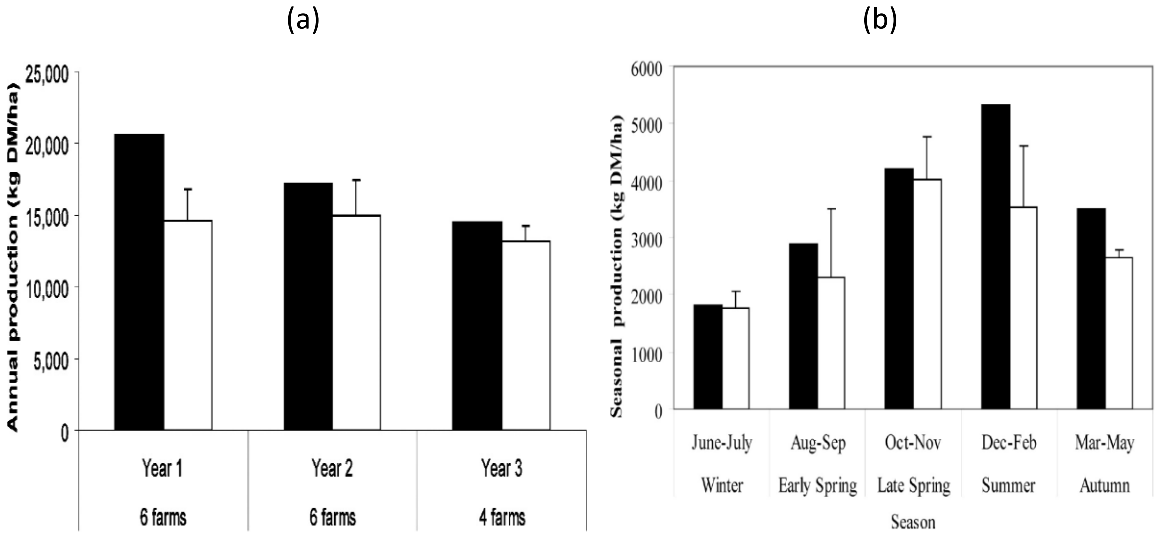

Figure 2.2 (a) Mean annual yields (kg DM/ha) of Tonic plantain based pastures and perennial ryegrass based pastures and (b) Mean seasonal distribution of dry matter production on four farms over 3 years from perennial ryegrass-based pastures and Tonic plantain-based pastures in Northland. Bars represent least significant difference (P<0.05).

Diverse species (mixed pastures of perennial ryegrass, white clover, prairie grass, lucerne, chicory and plantain) were similar with perennial ryegrass-white clover pastures in terms of DM production (14.7 t DM/ha/year *vs*. 15.3 t DM/ha/year respectively) in Waikato region (Woodward *et al*., 2013). Nobilly *et al*. (2013) showed that diverse pastures containing plantain and chicory produced 1.62 t DM/ ha more than the simple pastures (ryegrass-white clover) (16.8 *vs*. 15.5 t DM / ha) and concluded that this difference was due to the effect of these herb species. Notable growth in the diverse pastures was observed in summer when the herbs grew faster than ryegrass-white clover.

2.4 Chemical composition and biological compounds in plantain

2.4.1 Biological compounds in plantain

Plantain contains biological compounds (bio-active glycosides and condensed tannins) that can enhance or inhibit rumen functions (Table 2.1) which perennial ryegrass-white clover does not have. The bio-actives in plantain are the iridoid glycosides aucubin and catalpol (Deaker *et al*., 1994; Grigore *et al*., 2015; Navarrete *et al*., 2016; Tamura & Nishibe, 2002) and a phenylethanoid glucoside acteoside (Tamura & Nishibe, 2002). Navarrete *et al*. (2016) quantified the concentration of bio-active catalpol, aucubin and acteosides of plantain cv. Ceres Tonic over two periods (December & May and October, January & May). The levels of aucubins were 1.78-3.80 and 0.44-6.87mg/g DM and acteoside 23.6-35.4 mg/g DM and 0.5-41.7 in the first and second period respectively. These results were lower than 10-27 and 15-41mg/g DW aucubin and acteoside respectively reported by Tamura and Nishibe (2002) after evaluating the same cultivar. In both studies the levels of catalpol were low with Tamura and Nishibe (2002) reporting 1-2% of DM and Navarrete *et al*. (2016) recording 0.001-0.006% of DM.

2.4.2 Plantain effects on rumen activities

Plantain has antimicrobial compounds that can change the volatile fatty acid composition and inhibit rumen fermentation that could reduce bloat. In a study that evaluated the effect of bio-actives catalpol, aucubin and acteosides of plantain cultivar Ceres Tonic on rumen *invitro* fermentation, Navarrete *et al*. (2016) reported that aucubin reduced ammonia (NH_3) due to its bacteriocide activity, whilst acteosides increased gas production and acted as an energy source. Therefore, the conclusions of the study were that acteosides would be more beneficial to livestock production than aucubins.

Table 2.3 Biological / chemical composition of plantain and effects on grazing animals (adapted from Deaker *et al*. (1994), Grigore *et al*. (2015) and Tamura and Nishibe (2002))

Biological/ chemical compounds	Action	Authors
Antimicrobial compounds	Interacts with fermentation process and inhibits rumen fermentation.	Deaker *et al*. 1994 b
	Delayed silage fermentation with pH maintained above 5, less lactic or acetic acid produced. Insignificant degradation of protein to ammonia.	Isselstein, 1993a & 1993b
	Change microbial activity, lower gas production and reduce the severity of bloat	Katz *et al*. 1986
	Can promote the propionic producing bacteria and result in increased propionic to acetic ratios leading to increased milk production. Contains starch that can also aid the latter process.	Davey 1965; Forbes & France 1993 Stewart 1996
Medicinal properties	Anthelmintic properties; mild inhibition of *Trichostrongylus colubriformis* larvae suggested to be the effect of the iridoid aucubin glycoside activity.	Deaker *et al*. 1994 b
Mucilage or polysaccharide hydrocolloids	As it hydrates with water, mucilage form viscous jelly that acts as laxative and has purgative effects on the digestive system. Plantain contains 0.8% mucilage and is used commercially to control diarrhoea in calves.	Duke 1992; Brautigam & Franz 1985
Sugar alcohol sorbitol (D-glucitol)	Sorbitols present at about 2% in plantain and can accumulate in droughts. Enhances palatability as may contain 60-70% sweetness of sucrose.	Lewis 1984; Oku 1992
Tannins	When assessed through butanol-HCl test plantain contain about 0.4 to 1% condensed tannins (CTs). The CTs are not as high as in other herbs.	Terrell *et al*. 1992; Dorfer & Roselt 1989; Launert 1984
Mineral content	Contains high levels of minerals: Ca, Mg, Na, P, Zn that is associated with diuretic tendencies in animals ingesting plantain	Stewart 1996

2.4.3 Diuretic and anthelmintic effects of plantain

Iridiod glycosides (aucubin and its derivatives) are biological active compounds that contribute to diuretic and laxative effects on grazing animals (Tamura & Nishibe, 2002; Totty *et al*., 2013). Past research reported that the high mineral content and presence of iridiod catalpol in plantain were drivers of diuretic tendencies in animals. In sheep grazing plantain and perennial ryegrass-white clover, O'Connell *et al*. (2016) produced evidence that plantain has a diuretic effect. Sheep on plantain produced higher urine volume by 1.7 L on the first day of the trial than those on ryegrass-white clover and 0.5 L more on 5 subsequent collection days. In another study plantain-based pastures resulted in heavier and enlarged lamb kidneys due to large intake of water owing to the higher mineral contents of the herb (Stewart, 1996) and iridoid glycoside catalpol that has diuretic tendencies (Deaker *et al*., 1994). This indicates that catalpol or secondary plant compounds and mineral content that induce diuretic tendencies in grazing animals could be manipulated to increase urination frequency and spreading N patches in pastures (Edwards *et al*., 2015).

2.4.4 Plantain as an anthelmintic

The herb has anthelmintic properties that have been found to reduce faecal egg counts in sheep but not significantly to recommend it as a commercial anthelmintic (Judson, McAnulty, & Sedcole, 2009b). It is expected that future breeding programmes will objectively concentrate on concentrating anthelmintic properties to improve on the mild chemicals with current cultivars.

2.5 Nutritional value of plantain

2.5.1 Chemical composition and nutritional value of plantain and perennial ryegrass-white clover

The nutritional status of plantain is not well established (Sanderson *et al*., 2003) as most of the research carried out has focussed on medicinal characteristics. The latter authors investigated the nutritive value of plantain over two seasons in the USA under similar climatic conditions as those New Zealand. The average *in-vitro* true digestibility (IVTD) was 765g /kg which was similar to findings (698-913 g/kg) by Derrick, Moseley, and Wilman (1993). The latter authors study's average NDF was 465g/kg and considerably higher than 250g/kg DM reported by Wilman and Riley (1993). Crude protein averaged 141g/kg DM (Sanderson *et al*., 2003). The chemical composition of plantain and perennial ryegrass-white clover is shown in Tables 2.4 and 2.5 respectively. These studies compared the mineral and nutritive value of plantain and ryegrass-white clover (Harrington, Thatcher, & Kemp, 2006), described the grazing behaviour of dairy cows grazing different proportions of plantain (Gregorini, Minnee, Griffiths, & Lee, 2013), compared milk production and urine N concentration of lactating cows offered perennial ryegrass-white clover and pure plantain (Box *et al*., 2016) and evaluating the milk production and urinary behaviour of mid lactating cows grazing diverse or perennial ryegrass-white clover (Edwards *et al*., 2015). The averages from these different publications indicate some nutritional dissimilarity between perennial ryegrass-white clover and plantain. Crude protein is higher (252g/kg DM) in plantain than in ryegrass-white clover (225.5g/kg DM). Other values (g/kg DM) that make plantain more superior than pasture include a lower NDF (256.2 *vs.*421.7) and higher OMD (730.5 *vs.*657) (Tables 2.4 and 2.5 respectively). The higher mineral content from plantain reflects the higher ash in plantain (125.1g/kg DM) than in pasture (84g/kg DM)

Table 2.4 Chemical composition and nutritive value of plantain: Crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD), water soluble carbohydrate (WSC), metabolisable energy (ME) and Ash

CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (g/kg DM)	WSC (g/kg DM)	ME (MJ ME/kg DM)	Ash	Publication
283	256					119	Harrington *et al*. (2006)
298.9	148.3	188.7	740	85.5	12.5	143.4	Navarrete *et al*. (2016)
204	262	281	721	127	11.5	113	Gregorini *et al*. (2013)
223	224	299		109	11.4		Box *et al*. (2016)
252	222.6	256.2	730.5	107.2	11.8	125.1	Average

DM= dry matter

Table 2.5 Chemical and nutritive value of ryegrass-white clover: Crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD), water soluble carbohydrate (WSC), metabolisable energy (ME) and Ash

CP (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	OMD (g/kg DM)	WSC (g/kg DM)	ME (MJ ME/kg DM)	Ash	Publication
283	256						Harrington *et al*. (2006)
186	245	381		202	11.8		Edwards *et al*. (2015)
188	296	455	*657	75	10.5	*84	Gregorini *et al*. (2013)
233	282	429		76	11.2		Box *et al*. (2016)
222.5	269.75	421.7	657	117	11.2	84	Average

*Figures were not averaged
DM= dry matter

2.5.2 Minerals and dietary cation-anion difference

Plantain contains higher concentration of macro (P, K, Ca, Mg, Na) and micro (Bo, Cu, Mn, Zn) than perennial ryegrass (Sanderson *et al*., 2003). Besides contributing to diuresis, minerals play an important role in metabolic disorders like milk fever. The ability of cows to mobilise Ca from the bone is affected by acid-base balance that involves the dietary cation-anion difference (DCAD) estimated as (Na + K)-(Cl + S) in the diet (Judson & McFarlane, 1998). Whilst ryegrass-white clover pasture contains higher levels (>5mg/kg DM) of K that increases DCAD in lactating cows leading to hypocalcaemia (Sanderson *et al*., 2003), Rugoho *et al*. (2016) reported reduced DCAD for plantain and rape mix compared with ryegrass pasture (171 *vs*. 258 mEq/kg DM respectively).

2.6 Milk production

Most of the previous research that evaluated the effects of plantain on milk production used diverse pastures (Edwards *et a*l., 2015; Totty *et al*., 2013; Woodward *et al*., 2013) with limited studies on monoculture plantain or spatial separated plantain-perennial ryegrass-white clover (Box *et al*., 2016). Woodward *et al*. (2013) reported similar milk solids between the standard (perennial ryegrass-white clover) and diverse (chicory, plantain and lucerne) pastures except in autumn when standard pastures had greater results (1.48 *vs*. 1.29 kg MS/cow/day) due to different crude protein (CP) of 18.4% *vs*. 13.9% for standard and diverse pastures respectively (Table 2.5). In a related study, Edwards *et al*. (2015) reported similar milk solids for diverse and ryegrass-white clover pastures (2.09 *vs*. 1.94kg kg MS/cow/ day, respectively). Conclusions from these studies were that the diverse pastures maintained milk production at the same levels with cows that grazed perennial ryegrass-white clover with the greatest output being the reduction of urinary N to the environment.

Table 2.6 Milk production (kg/cow/day) form cows grazing [1]Mixed and [2]Standard pasture. There was no milk measurement in autumn year 3 since all cows on the farm had been dried off by the time of milk measurement.

	Year 1 (2010-11)			Year 2 (2011-12)			Year 3 (2012-13)	
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer
Mixed	18.6	11.6	9.6	18.6	15.4	12.0	23.0	18.2
Standard	19.7	11.6	8.1	18.6	15.0	12.2	22.4	18.4
SED	0.66	0.78	0.35	0.50	0.47	0.31	0.44	0.33
P value	NS	NS	*	NS	NS	NS	NS	NS

*P<0.05, NS= not significant
[1]Mixed pastures= perennial ryegrass +white clover + prairie grass + lucerne + plantain
[2]Standard pasture= perennial ryegrass + white clover

Totty *et al*. (2013) evaluated the effects of diverse pastures mix on DMI, milk yield and N partitioning in lactating cows and found that milk yield was higher (16.9kg/day) in diverse pastures (high sugar ryegrass and white clover with plantain, chicory and lotus) than traditional ryegrass-white clover pasture (15.2kg/day) and higher sugar ryegrass pasture (14.7 kg/day). In the same trial, DMI and N intake was not different between the different pastures with an average of 14.3 kg DM/cow/day and 583 g N/cow/day respectively that could have explained the similar milk solids among the treatments. There was a tendency for greater milk protein percentage in diverse pastures and therefore an increase in N use efficiency of 20.4% from diverse than 17.8% and 16.7% for the ryegrass-white clover and high sugar ryegrass-white clover pastures respectively. Gregorini *et al.* (2013) reported a DMI of 14.0 kg/cow/day for ryegrass and 16.3, 15.3 & 16.1 kg DM/cow/day for monoculture proportions of 20, 40, and 60% plantain respectively that had a slight edge above that of ryegrass control but not statistically different. Although this study did not measure milk production parameters it could be inferred that the extra energy from proportions with plantain would be directed into milk production. Box *et al*. (2016) reported higher milk solids for cows grazing plantain (1.67 kg MS/cow/day) than those grazing perennial ryegrass-white clover pastures (1.50 kg MS/cow/day) with those grazing 50:50 pasture–plantain in between the latter and the first (1.60 kg MS/cow/day).

2.7 Effects of plantain on N excretion in urine

Plantain contains biological compounds (bioactive glycosides and condensed tannins) that can enhance or inhibit rumen functions (Deaker *et al*., 1994). Reduction of N in urine and milk is attributed to secondary bio-actives aucubins and acteosides that reduce *invitro* NH_3 gas production (Navarrete *et al*., 2016). Plantain has been evaluated for their contribution to reduce N excretion in pastures in New Zealand.

Diverse pastures that include plantain, chicory and lotus have the potential to mitigate some environmental challenges resulting from sheep or dairy intensification. The inclusion of plantain, lotus and chicory to ryegrass-white clover pastures reduced the dairy cows' urinary N excretion by 17.3% compared with the urine of those grazing high sugar ryegrass-white clover (353.8 g/day *vs*. 426.6 g/day respectively) (Totty *et al*., 2013). In this trial it was postulated that the partitioning of N to milk production was due to the condensed tannins in the herbs. When Cheng *et al*. (2015) measured urinary excretion on heifers grazing ryegrass-white clover (PA), chicory (CH), PA +CH, plantain (PL) and PL + PA they reported no difference in urinary nitrogen and or urinary output per heifer amongst the treatments (Table 2). The higher urination frequency (times/6 hours) in PA + CH and CH (PA+ CH= 6.2; CH= 4.5) than in other treatments (PA= 3.1; PA+ PL= 2.8; PL= 2.9) was attributed to diuretic effects of high mineral containing chicory (potassium, calcium, sodium) than the grass; however this did not explain why plantain that also has high mineral content (calcium, magnesium, sodium, phosphorus, zinc) (Stewart, 1996) had lower urination frequencies. Calculated water intakes from plantain (42 litres) and chicory (55 litres) were higher than in other treatments. The variation in urination frequency could not be explained because drinking water intake was not physically recorded. O'Connell *et al.* (2016) using sheep in metabolism stalls produced evidence that plantain has a water diuretic effect. Sheep on plantain produced higher urine volume by 1.7 L on the first day of the trial than those on ryegrass-white clover and 0.5L more urine on 5 subsequent collection days.

Table 2.7 Grazing and urination behaviour during the first six hours of fresh herbage allocation and spot sample measured urinary nitrogen concentration, estimated urinary nitrogen (N) excretion and urinary output of heifers grazed on 100% pasture (PA), 100% chicory (CH), 100% plantain (PL), 50% pasture + 50% chicory (PA + CH), and 50% pasture + 50% plantain (PA + PL) (Source: Cheng *et al*. 2015)

	CH	PA+ CH	PA	PA+ PL	PL	LSD	P value
Grazing (mins/6hrs)	255	249	226	240	245	24.5	0.22
Idling (mins/6hrs)	93[a]	89[a]	63[b]	90[a]	90[a]	20.8	0.032
Ruminating (mins/6hrs)	12[c]	23[bc]	71[a]	30[b]	25[bc]	16.2	<0.001
Bite rate (times/min)	23	28	34	34	33	9.46	0.076
Urination (times/6hrs)	4.5[b]	6.2[a]	3.1[c]	2.8[c]	2.9[c]	1.18	<0.001
Urinary N excretion (g/day)[1]	67.0	74.2	69.8	72.4	79.9	14.72	0.423
Urinary output (kg/day)[2]	59.3	66.4	65.3	61.1	51.2	19.11	0.499
Urinary N concentration (%)	0.19	0.28	0.21	0.24	0.28	0.107	0.351

[1]Estimated from plasma urea nitrogen and liveweight (Kohn, Dinneen, & Russek-Cohen, 2005). [2]Estimated by creatinine and liveweight (Chizzotti, Valadares Filho, Valadares, Chizzotti, & Tedeschi, 2008). [a-b] Means within the same row with different superscripts differ (P< 0.05).

Urinary N concentration of late lactating cows grazing perennial ryegrass-white clover pastures and 50:50 plantain-ryegrass, was 33% lower in the latter (3.6g N/L) than ryegrass-white clover pasture (5.4g N/L) (Box *et al*., 2016). Consistent with this report, Edwards *et al*. (2015), found a urinary N concentration 20% lower for cows grazing diverse (4.9g N/L) than those on ryegrass-white pastures (6.1g N/L). Urinary N was about 32% less for heifers offered plantain baleage (0.36% N) than those on perennial ryegrass-white clover baleage (0.53% N) (Judson & Edwards, 2016). The mechanism by which plantain reduces N in urine is not clear. Reduced intake of N in plantain could not be the reason as the latter authors' plantain baleage N was numerically higher than that in pasture but still there was large difference (1.7g N/L) in urine excretion.

2.8 Water intake

Most studies on water intake have been carried out on cows fed total mixed rations (TMR) in stalls measuring drinking water (Cardot, Le Roux, & Jurjanz, 2008; Castle & Thomas, 1975; Jago, Roche, Kolver, & Woolford, 2005). The range of water intake in these studies was 49.9-83.6 L/day/cow. In pastures, Morris, Hickey, Thom, and Waugh (2010), reported a total water intake (from drinking water and pasture) of 104-110.6 L/cow/day on pasture. Due to its chemical bio-actives and minerals, plantain was reported to have diuretic effects encouraging cows to drink more water (Stewart, 1996). Edwards *et al*. (2015) did not find a difference in drinking water intake in cows grazing diverse (plantain, lucerne, chicory, ryegrass/white clover) and simple pastures (26 *vs*. 28 L/cow/day respectively) as expected. These authors estimated that the feed water intake was 100 L/cow/day.

2.9 Animal behaviour

2.9.1 Spatial monocultures and mixed species pastures

Livestock preference for plantain is not well established (Gregorini *et al*., 2013; Raeside *et al*., 2014) although this plant has shown ability to increase DM production in summer (Moorhead & Piggot, 2009; Powell *et al*., 2007) and improve sheep growth rates (Judson *et al*., 2009a). Traditional pastures in New Zealand consist of mixtures of pasture grasses and white clover, with the legume in low proportions of 5- 15% (Marotti, Cosgrove, *et al*., 2002). Mixed species pastures have limitations that include; encouragement of search patterns by livestock that leads to exploitation of other species and areas in the paddock relative to the total area (Distel, Soca, Demment, & Laca, 2004). This essentially explains that there is inter-specific competition between species with animal selective trends further reducing the preferred species proportion. Maintenance of species in mixed species pastures is thus complicated (Marotti, Cosgrove, *et al*., 2002). Monocultures in spatial arrangements have increased milk production and growth rates in sheep (Marotti, Chapman, Cosgrove, Parsons, & Egan, 2002).

The principles guiding spatial arrangements of monocultures are that the area and plant ratio in spatial distribution must be close to optimal diet for the grazing animal. Spatial arrangements of monocultures present advantages of choice of species without interspecies competition (Edwards, Parsons, & Bryant, 2008). This was demonstrated in a trial when sheep grazed spatial monoculture of ryegrass and subterranean clover resulted in improved species persistence with apparent lamb and ewe growth being 20-30% and 30- 110% faster in adjacent monocultures respectively than mixes (Venning, Kearney, Chapman, & Thompson, 2004). Spatial separation of perennial ryegrass and clovers has been demonstrated to increase milk production by ~10% in dairy (Marotti, 2005; Rutter, Young, Cook, & Champion, 2003) and increase sheep growth rates by ~25% (Cosgrove, Hyslop, Anderson, Litherland, & Lambert, 2003; Venning *et al*., 2004). The literature cited above all pertains to ryegrass and clover pastures and therefore plantain- grass arranged spatial pasture experiments in dairy systems are limited.

Raeside *et al*. (2014) compared pure swards of ryegrass, plantain and a 50:50 plantain ryegrass spatial pasture on ewe performance from mid pregnancy to lamb weaning. There were no clear differences in the growth of the ewes (Table 2.6) and the lambs and faecal egg count (FEC) reduction. There is a possibility of having had significant differences if the ratios of the spatial mixes were varied.

Table 2.8 Lamb liveweights (kg) at birth, marking, and weaning, showing differences between single and twin lambs on perennial ryegrass (PR), plantain (PL), or a perennial ryegrass-plantain spatial mixture (PR + PL)

Treatment	Pregnancy status	Birth (14 Sept- 19 Oct. 2010)	Marking (27 Oct. 2010)	Weaning (5 Jan. 2011)
PR	Single	5.25	16.90	35.57
	Twin	3.95	13.27	25.71
PL	Single	5.57	15.15	29.90
	Twin	4.58	13.75	27.97
PR + PL	Single	5.14	16.14	32.07
	Twin	4.33	13.13	25.95
*LSD		1.26	1.97	5.44

*LSD (*P*=0.05) values show the pasture treatment x birth type interaction at each weighing (Source: Raeside *et al*. (2014)).

According to Marotti *et al*. (2001) dairy cows grazing spatial monocultures of ryegrass and white clover had higher total daily intake (21 kg/ day) than the those grazing mixed species pastures (18.6 kg/day). The spatial monocultures of the same species indicated increased milk production by 11% when compared with mixed species and 28% more when compared with ryegrass alone. In these studies the proportions considered (ryegrass: white clover) were 50%:50% without lower proportions to show the progression of response to treatments from lower to higher proportions.

2.9.2 Grazing behaviour

Animal grazing behaviour is affected by external factors (e.g. the plant characteristics) (Cosgrove & Edwards, 2007). Cheng *et al*. (2015) reported that rumination time of dairy heifers was lower when grazing chicory and plantain than ryegrass/white clover for the dairy heifers. This was attributed to the low NDF (245 ± 48.3 g/kg DM and 240 ± 25.5 g/kg DM for chicory and plantain respectively versus 376±17.9g/kg DM for ryegrass/white clover) and high mastication activity reducing forage particle size. In the trial a lower bite rate was observed for chicory (23 bites/minute) than for plantain (33 bites/minute) and ryegrass/white clover (34 bites/minute). A lower bite rate for chicory could have been caused by a higher leaf mass in the mouth that would take longer mastication.

Gregorini *et al*. (2013) recorded similar behavioural results when they compared proportions of chicory (20, 40, and 60%), plantain (20, 40, and 60%) and ryegrass-dominant control on dairy cows. For both plantain and chicory treatments there was a tendency for the cows to be more idle especially with 60% proportion of both herbs. Cows in the 60% plantain treatment ruminated 90 minutes less than those on ryegrass and masticated about 3 times when compared with the control. As rumen function is related to ingestive mastication, particle breakdown and size swallowed (Anil & Forbes, 1988; Rattray, Brooks, & Nicol, 2007), herb breakdown results in faster rumen turn over and fermentation rates (Gregorini *et al*., 2013). This was also aided by the lower NDF for chicory and plantain (22.2 & 28.1% respectively) than ryegrass (45.5%) making mastication easier and therefore less rumination time.

2.9.3 Urination behaviour

The urine volumes in lactating and non lactating cows vary considerably. Edwards *et al*. (2015) recorded 1.8-2.4 litres of urine /urination event in lactating cows grazing diverse (chicory, plantain, lucerne) and ryegrass-white clover. The urine volume/urination event was similar between the diverse and the simple pastures (2.2 *vs*. 2.0 L). When Ravera *et al*. (2015) evaluated the urine volumes on non lactating cows on kale and fodder beet, they also reported varying volumes (<1 -5 litres/urination event and 8.7-47 litres/24 hrs/cow), but in this case the total urine volume/cow/24hrs was different (30 *vs*. 18 litres respectively). The urine volume variability would make it difficult to estimate average N leakage in a urine patch without confirming with other measurements e.g. lysimeters data from the paddocks (Edwards *et al*., 2015).

Many studies have measured urination frequency in livestock (e.g. Clark *et al*. (2010), Ravera *et al*. (2015), Edwards *et al*. (2015) and Robichaud, de Passille, Pellerin, and Rushen (2011)). Ravera *et al*. (2015) and Farrell (2015) reported urination frequencies 8-12 and 8.5-10.3 per 24 hours on kale and fodder beet forage respectively whilst Edwards *et al*. (2015) found 11.6-15 frequency/24hours on lactating cows grazing ryegrass-white clover and diverse pastures. In the latter trial, the diuretic effects of diverse pastures (Navarrete *et al*., 2016; Tamura & Nishibe, 2002) increased urination frequency/ 24hours as expected (15 for diverse *vs*. 11.6 for ryegrass-white clover). Clark *et al*. (2010) confirmed similar results (14.1 urinations/day) for cows grazing pasture for different hours/ day and 13.03 urinations for late lactating cows under similar pasture regime, whilst Robichaud *et al*. (2011) reported a urination frequency that varied from 2-19/day on lactating cows fed total mixed rations (TMR) in stalls.

On evaluating if urine patch area was related to feed treatments Ravera *et al*. (2015) and Farrell (2015) recorded 0.47 & 0.25m^2 and 0.22 & 0.25m^2 for kale and fodder beet, respectively under wintering pregnant cows. The literature on effect of plantain based pasture on urine patch size could not be found.

2.9.4 Summary

Research on the complementarities of alternative herbs with traditional pastures and their effects on animal performance is still inconsistent and inconclusive. This is because there is variation in scientific evidence on integration of pastures to improve animal production and reduce environmental pollution

Plantain and other herbs have been incorporated into pastures to form diverse pastures resulting in increased DM production and reduction of N excretion. While there is evidence supporting the positive effects of plantain on urinary N loss, there is no information on how much plantain is required to have an impact on N loss. Further, most studies on diverse pastures have included plantain in the mix. As nutrient management becomes increasingly regulated and monitored, the effect of one plant species in a mixed pasture will be difficult to estimate due to fluctuations in botanical composition caused by season and management. Data is therefore required to determine what proportion of the diet plantain should consist of to support high milk yield and reduce urinary N loss.

Chapter 3 Methods and Materials

3.1 Experimental site

The experiment was conducted from 11 to 20 April 2016 at the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand (43°38S', 172°27E', 17 m.a.s.l) in three 0.5 ha replicated paddocks with a combined area of 4.5 ha. The soil type was a free draining Templeton fine sandy loams with feldspar (32-43%), quartz (20%) and amorphous minerals (10-30%) (Bureau, 1969; Hewitt, 2010). The Lincoln University Research Dairy Farm experiences an average 36 days of screen frost with mean annual minimum and maximum temperatures of 4 ^{o}C and 32 ^{o}C, respectively and average annual rainfall of 666 mm / annum (SIDDC, 2016).

Prior to the experiment, the paddocks had been sown with a mixture of perennial ryegrass, tall fescue, high sugar grass, chicory and plantain. Land preparation and planting of pastures was conducted in January 2016. To remove the existing pastures and weeds, glyphosate was applied with a tractor mounted with a Bargam 15 m boom sprayer at a rate of 2 litres / hectare. The soil was then power-harrowed. Plantain (cultivar Tonic) and perennial ryegrass (cultivar Arrow AR 1)-white clover (cultivar Weka) mix were sown in strips using a coulter drill planter in the 3 paddocks. Seeding rate was 12 kg/ha for plantain and 24 kg/ha for perennial ryegrass-white clover. The perennial ryegrass-white clover seed ratio mix was 20 kg/ha of perennial ryegrass and 4 kg/ha of white clover. The width of the strips was adjusted so that within each paddock four 20m x 186m areas where sown in strips so that the area was made up of 20 m pasture (0% plantain), 17 m pasture, 3 m plantain (15% plantain), 14 m pasture, 6 m plantain (30% plantain), and 8 m pasture, 12 m plantain (60% plantain). These were allocated randomly to each paddock. To control broad leave weeds the paddocks were boom sprayed with bentazone at a rate of 3 litres/ha about 3 weeks after germination of pasture.

After sowing, the paddocks were irrigated using a centre pivot irrigator. Plant emergence dates were recorded and used to estimate days to physiological maturity and the start of the grazing trial. To maintain uniform growth, the pastures were lightly grazed with heifers and rolled to control broad leaved weeds, mainly fat hen (*Chenopodium album* L.) 3 weeks before the trial. Nitrogen fertiliser (Urea 46% N) was applied at 50 kg N/ha for both plantain and perennial ryegrass-white clover 30 days prior to the start of the grazing trial.

3.2 Animal ethics statement

All animal samples were collected in compliance with the Lincoln University Animal Ethics Regulations (Approval Number 2016-11).

3.3 Experimental design

The experimental design was a complete randomised design (CRD) with 3 replicates of 4 pasture treatments. Forty eight late lactating primi and multiparous Friesian x Jersey cows were blocked according to pre-experimental averages (mean, ± s.e.m) liveweight (499.21 ± 6.44 kg), milk solids (1.41 ± 0.02 kg), milk yield (14.14 ± 0.29 kg) and age (5.42 ± 0.19) and randomly allocated to one of three replicates of four treatments (0% plantain, 15% plantain, 30% plantain, 60% plantain).

3.4 Cow management

The cows were selected from the LURDF main cow herd that had grazed on ryegrass-white clover pasture prior to the experiment. The experiment consisted of a 3 day adaption period and a 10 day experimental period. In the adaptation period 12 cows in each treatment grazed as a group before they were allocated to the 3 replicates of 4 cows per replicate and allocated to the respective plots.

Cows were milked twice a day (at 0700 hrs and 14:00 hrs) with a 24 side herring bone automatic milking system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden) and were offered a pasture target allowance of 25 kg DM/cow/day above ground level. The allocation of pasture allowance was based on pre-derived calibration equations from random herbage quadrat cuts. To achieve this allowance daily, temporary electric fence was removed every morning with the allowance based on the rising plate meter (RPM) (Jenquip F150 Electronic Pasture Meter, Fielding, New Zealand) mass estimations with area used to control allowances after the morning milking, after a 24 hour break. The daily herbage allowance was back-fenced to stop animals grazing the re-growth residual. Water was provided adlib through portable troughs that were fitted to flow meters.

At day 7 and 8, two cows from a replicate of PL30 had bloat, thus water troughs in all treatments were treated with Bloatenz® at a rate of 50 ml per 500 kg cow liveweight. This condition did not however affect the overall production performance of the cows in that treatment.

3.5 Measurements

Data was collected on herbage (dry matter, quality) and animal variables (body condition scores, liveweight, milk production, urine, faeces and blood) and cow behaviour (grazing and urination).

3.6 Herbage dry matter and quality

3.6.1 Herbage mass

Herbage mass was assessed pre- and post-grazing on a daily basis using two methods: the rising plate meter (RPM) and herbage quadrat cuts. Thirty pre- and post grazing pasture height measurements were taken daily using a calibrated RPM. The pre-grazing pasture height measurements were taken in the area to be allocated for grazing the next day. Both the pre- and post grazing pasture height measurements were taken after milking at 0800 hrs daily. A total of 42 and 60 pre-grazing perennial ryegrass-white clover and plantain quadrats each 0.2 m^2 were cut to ground level 3 days before the experiment. Two RPM measurements were recorded before the herbage was harvested. The harvested herbage was oven dried at 60^oC until constant weight. Linear relationship between dry herbage weights and pasture height for plantain and perennial ryegrass-white clover was determined and best fit equations fitted to the data. The calibration equations for perennial rygrass-white clover was; herbage mass (kg DM/ha)= 79.1x + 987 (r^2=0.60) and plantain (kg DM/ha) =74.2x +335 (r^2=0.67), where x was the height of the pasture based on RPM in RPM (0.5 cm) units. Daily herbage mass allocations were estimated using the calibration equations.

On days 4, 6, 8 and 10 of the experiment two quadrat (each 0.2m^2) cuts per replicate pre- and post grazing were collected and dried as described earlier. The linear relationship between dry herbage and RPM herbage height was; herbage mass (kg DM/ha) = 94.3x+932.9 (r^2 =0.95) and 140.8x+324.4 (r^2 =0.95) for plantain and pasture respectively. The equations were used in calculating pre- and post-grazing mass to estimate dry matter intake during the experimental period.

3.6.2 Dry matter, quality, botanical composition and dietary cation-anion difference

A total of 10 pasture samples were taken from perennial ryegrass-white clover and plantain at grazing height pre- and post grazing and bulked over 3 days. The cuts were processed within 30 minutes after collection to avoid nutrient loss. Approximately 100g fresh weight (FW) from the bulked samples was washed, weighed and dried in an oven at 60^oC until constant weight (48 hours) and reweighed to determine dry matter (DM) percent. From the bulk, a further 100 g FW was separated into botanical components (white clover, ryegrass, plantain, monocotyledon weeds, dicotyledonous weeds and dead material) weighed and oven dried at 60^oC for 48 hours. Botanical composition was then determined on DM basis.

Another 100 g subsample was freeze dried and ground through 1mm sieve for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), DM digestibility (DMD), digestible organic matter in the dry matter (DOMD), DM, organic matter (OM), water soluble carbohydrates (WSC) analysis by near infrared spectrophotometer (NIRS, Model: FOSS NIRS Systems 5000, Maryland USA) with different prediction equations for grass and plantain, the coefficients for NIRS predicted as reported by Bryant, Gregorini, *et al*. (2012). The metabolisable energy (ME) was estimated from the equation:

MJ ME/kg DM = 0.16 x DOMD (CSIRO, 2007).

From the ground samples, an average of 8 g/sample was analysed for dietary cation-anion difference (DCAD) minerals (P, K, S, Ca, Mg, Na) through the nitric acid/hydrogen peroxide digestion followed by ICP-OES whilst chloride (Cl) was determined by NIR and 2% nitric acid extraction followed by potentiometric titration (Hill Laboratories, Hamilton, New Zealand). DCAD was estimated thus:

Dietary cation-anion difference (DCAD) (mEq/kg DM) = [(% Na x 434.98) + (%K x255.74)] - [(%Cl x 282.06) + (% S x 623.75)] for ryegrass white clover and plantain pastures plots (Lean & DeGaris, 2010) and then proportions used to estimate percent content in diet.

Iridoid glycosides (aucubins and catalpol) and phenylpropanoid glucoside, acteoside were determined from freeze dried ground plantain subsamples using a Dionex Ultimate 3000 High-Performance Liquid Chromatography (HPLC) auto-sampler and multi-wavelength detector as outlined by Tamura and Nishibe (2002). Four-hydroxybenzaldehyde was used as an internal standard to calculate recovery percentages for each sample.

3.7 Water intake

Standard clean water for animal drinking was provided *adlibitum*. A water flow meter (Arad M20) was inserted into each water trough to estimate the group daily water intake. Apparent drinking water intake per replicate was the difference in morning meter reading before cows returned from milking and the previous day's meter reading after moving the trough and refilling. Daily water intake from the feed was estimated as:

kg fresh herbage - kg dry matter intake (DMI); where kg fresh herbage = kg DMI/ dry matter %.

Total water intake was therefore the sum of drinking water intake and feed water intake.

3.8 Animal measurements

3.8.1 Milk yield and composition

Milk yield was measured with an automated milking machine (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden) every day during each milking period. Milk samples for the experimental phase were collected at each morning and afternoon milking on day 1, 3, 5, 7 and 9 of the experimental period. One sub sample was used to determine milk urea nitrogen (MUN) and was processed through methods outlined by Totty *et al*. (2013). The milk samples were centrifuged at 4000 x *g* for 10 minutes to solidify fat. The resultant skim milk was pipetted into a micro-centrifuge tube, chilled at -20^{o}C before analysis for MUN at Lincoln University Riddolls Laboratory (Christchurch, New Zealand) using an automated random access clinical chemistry analyser, RX Daytona Modular P analyser (Roche Hitachi, Basel, Switzerland) by an assay (Talke & Schubert, 1965). The second subsample was immediately sent to Livestock Improvement Corporation Limited (Christchurch, New Zealand) for milk composition (protein, fat, somatic cell counts) analysis. To analyse for these, the MilkoScan (Foss Electric, Hillerod, Denmark) was used.

3.8.2 Body condition scores, liveweight and feed conversion efficiency

Body condition scores (BCS) were determined at adaptation (day 0) and at the end (day 10) of the trial using the 5 point scoring system (Ferguson, Galligan, & Thomsen, 1994).

Live weight was recorded daily after the morning and afternoon milking sessions using a walk-over automatic scale. The initial (day 0) and the final (day 10) weights of the cows were taken off an electronic weighbridge scale for accuracy. Body condition scores and liveweight were collected to explain the energy partitioning and expenditure by the cows.

3.8.3 Urine, faecal and blood measurements

Urine and faecal samples were collected on day 3, 5, 7 and 9 of the experimental period after the morning and afternoon milking. Cows were handled in the veterinary handling facilities, rectally palpated and vulva stimulated to collect faecal and urine samples respectively according to methods of Totty *et al*. (2013). On days 3, 5, 7 and 9 of the experimental period, urine was collected, treated with sulphuric acid to a pH 4.0 to prevent volatilisation and then frozen at -20^{o}C until laboratory analysis was carried out. It was analysed for total N% using the N analyser (Vario MAX CN, Elementor Analysensysteme, Hanau, Germany). Urea, ammonia and creatinine were determined with a Randox RX Daytona analyser (Randox Laboratories Ltd., Crumlin, United Kingdom). Prior to acidification on days 8, 9 and 10 of the experimental period, pH of each urine sample was tested with a portable pH meter (Ezdo 7011 pH mV Temp). Purine derivatives (allontain and uric acid) were determined from urine subsamples using the HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as previously described (Czauderna & Kowalczyk, 2000) with modifications by George *et al*. (2006). The estimations of microbial N were based on purine derivatives and metabolic weight (Singh *et al*., 2007; Totty *et al*., 2013).

Faecal samples collected on days 3, 5, 7 and 9 of the experiment were stored at -20^{o}C and then thawed and two subsamples were separated. One sample was oven dried at 100^{o}C for 12 hours to determine DM%. The other sample was freeze dried, ground and analysed for DM% and total N% as illustrated by Totty *et al*. (2013).

Blood was collected from the coccygeal vein using 10 mL sodium heparin tubes (Greiner Bio-one, Kremsmitter, Austria) and cooled on ice before centrifugation at 3000 x *g* at 4^{o}C for 15 minutes. The separated plasma was stored at -20^{o}C before analysis for creatinine and urea N concentration as in Totty *et al*. (2013).

3.9 Cow behaviour

3.9.1 Feeding, rumination, bite rate and idling

Physical observations for rumination, grazing, idling were conducted on days 2, 4 and 8 (day 6 observations were disrupted by rain). All cows were scanned for all activities with 2 focal cows per replicate being observed for time taken for 10 bites per herbage pasture species. Observations were done for 2 hours after the morning milking and 2 hours after the afternoon milking.

3.9.2 Urine quantity and urination frequency

Frequency of urination and urine volume was measured using the urine harness and sensors developed under Animal Ethics Committee (AEC 551) on days 4-10 with at least 1 cow per treatment allocated randomly. A urine harness consisted of a flow meter that was attached to the cow's vulva by a rubber hand glove using Loctite Super Glue (IDH number 1363131) with a data logger on the shoulder harness pocket (Ravera *et al*., 2015). As the cow urinated onto the flow meter, data messages of urine volume and frequency were relayed to the data logger and stored in a central computer (Edwards *et al*., 2015). Skin conditions in areas of attachment of the cow and harness were monitored in event of injuries and the harness was only allowed to be used for 24 hours on each randomly selected cow.

Initially 31 cows were fitted with urine harnesses, with 24 animals' data remaining after the harnesses fell off or the flow meters failed to download data. Of the 24, 14 cows' data remained after further cleaning for too frequent urination bouts and or abnormal urinations.

Urine patch area was measured over 3 days (day 8, 9 and 10 of the experimental period) either at 0500hrs before the cows were collected for milking or in the evening (from 1700hrs) using FLIR infra red imaging thermal camera. The process involved watching out for the cows that urinated, recording the pasture type (plantain or ryegrass-white clover) and taking photographs urine patch. A 30cm ruler was used as a scale. One hundred and sixteen urine patches were photographed from the 12 paddocks. The average number of urine patches photographed was 29/treatment.

To validate the actual urine patch area, a calibration curve was developed by evaluating the relationship of known quantities of water and the area wetted by the water in the paddocks. Calibration measurements consisted of 10 volumes increasing by 0.5litre (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5L) replicated at least 8 times per volume for plantain and perennial ryegrass-white clover pastures. Warm water was gradually poured at cow vulva level in the evening and the pictures of the wetted area captured by a FLIR infra-red imaging thermal camera. A 30cm ruler was used a scale unit. The calculation of the urine patch and calibration images area was performed using SketchAndCalc^{TM} processing software.

3.10 Weather

Weather data was collected on site from the Lincoln University micro-satelite station during the experiment. Daily ambient temperatures (minimum, maximum and mean) and rainfall were recorded.

3.11 Calculations

3.11.1 Dry matter intake

To examine dry matter intake (DMI), the daily RPM herbage height readings pre- and post-grazing were taken. Apparent DMI was determined by the difference between pre and post grazing mass thus:

DMI (kg DM / cow/day) = ((pre-grazing - post grazing residual) x area grazed))/ number of cows

The dry matter intake of the composition of feed was estimated through processes explained by Dalley, Roche, Grainger, and Moate (1999) listed below:

i) Chemical composition of nutrients offered= ((Proportion mass of grass x nutrient concentration %)+(Proportion mass of plantain x nutrient))/Total grass and plantain mass.

ii) Nutrient selection = ((pre-grazing mass x pre-grazing nutrient concentration %)-(post-grazing mass x post grazing nutrient %))/(pre-grazing mass – post-grazing mass)

Nutrient selection differential was expressed as the concentration of consumed nutrients as a ratio of the pre-grazing herbage nutrient concentration. More nutrients would be selected for when the selection was more than 1, no selection when the differential was 1 and less selection for less than one.

iii) Selection differential = Selected nutrient / pre-grazing nutrient

iv) Nutrient or mineral intake= Pre-grazing total nutrient or mineral x selection differential x DMI

v) Concentration of nutrient or mineral ingested = Nutrient or mineral intake/DMI

3.11.2 Feed conversion efficiency

The feed conversion efficiency (FCE) for individual cows for milk solids ($_{ms}$) and for milk yield ($_{my}$) was calculated as follows:

a) FCE $_{ms}$ = ((MS yield (kg/ cow group/ day)) / ((DMI (kg DM/cow group/day))

b) FCE $_{my}$ = ((MY (kg/cow/day)) / ((DMI (kg DM/cow/day))

These estimations are based on back calculations involving milk yields or milk solids, liveweight, DMI and ME as described by Sheahan, Kolver, and Roche (2011).

3.11.3 Microbial N supply

Microbial N supply estimations were based on purine derivatives and metabolic weight (Singh *et al*., 2007; Totty *et al*., 2013) where;

i) Total purine derivatives (PD)= allontoin + uric acid

ii) PD index =[(total PD mmol/L)]/creatinine (mmol/L) x $BW^{0.75}$; where creatinine was extrapolated from an equation by Pacheco, Lowe, Burke, and Cosgrove (2009)

iii) Daily excretion of PD (dPD mmol/L of $BW^{0.75}$)= PD index x 0.9

iv) Purines absorbed daily were estimated as: daP=[dPD (mmol/kg $BW^{0.75}$)- 0.385 x $BW^{0.75}$]+ 0.85

v) Microbial N (g of N/d) = (daP x 70)/ (0.116 x 0.83 x 1000)

vi) Urinary N excretion (urinary g of N/day)=21.9 (mg/kg) x BW (kg) x [1/urinary creatinine (mg/kg)] x urine N (g/kg) as described by Pacheco et al. (2009).

3.11.4 Nitrogen loading

The calculation of nitrogen loading was based on formulas by Farrell (2015). Nitrogen loading (kg/ha) =(average urination event volume x urine nitrogen concentration)/ (urine patch area)

3.12 Statistical analysis

3.12.1 Data entry

Data was entered in Microsoft Excel (2010) for collation and manipulation before analysis in GenStat (Release 16, VSN International Ltd).

3.12.2 Statistical analysis

Normality and equality of variances across treatments were tested before data was subjected to analysis of variance (ANOVA) in GenStat (Release 16, VSN International Ltd) to test effect of proportion of plantain. The statistical significance was declared at F-probability of 0.05 and 0.01 and the least significant difference (LSD) at 5%. Desired power of the experiment was 80%; with data in the analysis from milk solids (kg/cow/day), raw coefficient of variation (CV) =13% (Mackle, Bryant, Petch, Hill, & Auldist) and variation in milk composition of milk protein from pasture based fed pasture in late lactation being CV =7.5%.

Analysis of animal data (milk, urine, faeces) was based on the mean of 4 cows per replicate with data average across days prior to analysis. Herbage data (mass, botanical composition, quality) was analysed by ANOVA with plot as replicate and data averaged across measurement days prior to analysis. Urination behaviour was analysed for urine volume and frequency. The latter measurements (n=14) were analysed by unbalanced ANOVA using Genstat regressions because the replications were not balanced in the treatments. Each cow's urine volume or urine patch record was regarded as a replicate.

Data was tested for ante-dependance structures to determine that the repeated measurements were more appropriate as a data collection method.

Chapter 4

Results

4.1 Weather

The weather data is shown in Figure 4.1. Mean temperature for the experimental period was 11.9^{o}C and the mean maximum and mean minimum temperatures were 19.6^{o}C and 4.8^{o}C respectively. The rainfall on day 2 and 8 of the trial was approximately 2.4 mm.

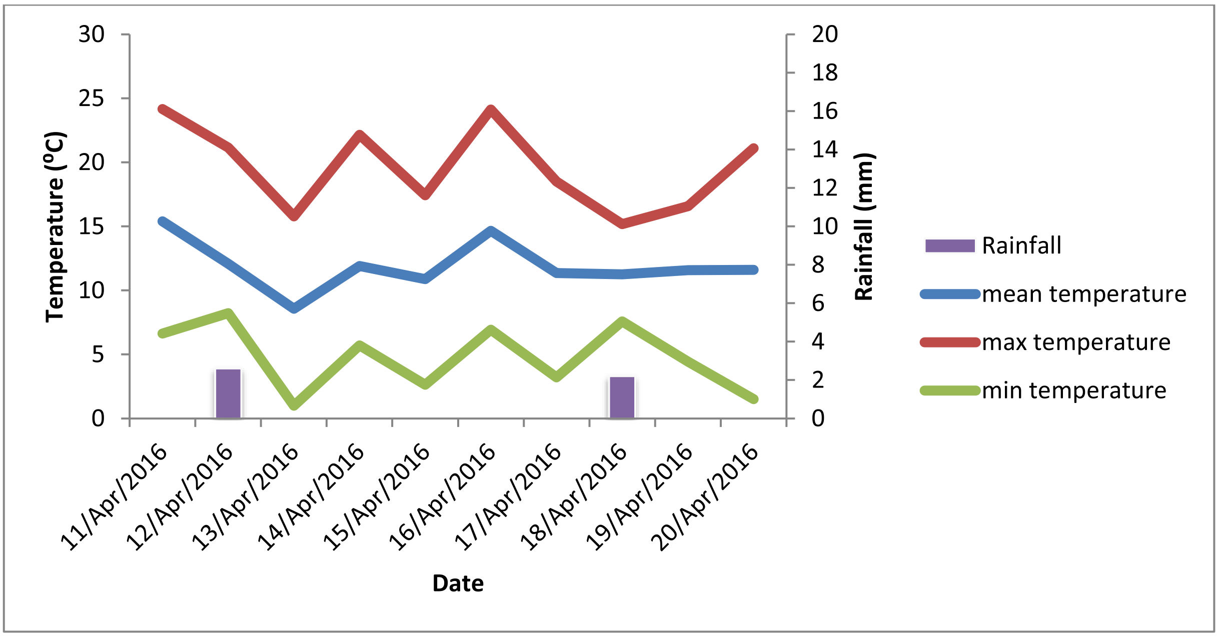

Figure 4.1 Daily ambient temperature and total daily rainfall at LURDF during the experimental period

4.2 Herbage characteristics

4.2.1 Herbage mass

The estimated pre- and post-grazing herbage (kg DM/ha) mass of perennial ryegrass-white clover and plantain based on RPM measurements is shown in Table 4.1. Pre-grazing pasture and plantain herbage mass (kg/ha) averaged 3858 and 4973 respectively with monoculture herbage mass similar amongst the treatments. The average post-grazing mass for ryegrass-white clover and plantain were comparable (1505 *vs*. 1511 kg DM/ha), with plantain post grazing mass increasing (P=0.001) from PL15 to PL60, whilst pasture in PL0 was grazed to a similar mass as that in PL30 and PL60 but different to PL15 (P=0.049). Pasture height (cm), for pre- and post-grazing ryegrass-white clover (average 25.1 and 8.4 respectively) and pre-grazing plantain (average 42.8 cm) was the same between the treatments. The cows left more plantain herbage as the proportion of plantain increased in the diet (*P*=0.001), but consumed pasture lower into the ground as the proportion of plantain increased (*P*=0.05)

Table 4.1 Total DM (kg/ha) of pre- and post-grazing herbage mass and compressed pasture height of perennial ryegrass-white clover (Gr) and plantain (PL) grazed by late lactating cows in autumn (*P*- value for comparison of pastures within treatments (Gr) and plantain (PL) within treatments are are shown). PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain

	Treatments										
	PL0	PL15		PL30		PL60		SEM	*P*-value	SEM	*P*-value
Variates	Gr	Gr	PL	Gr	PL	Gr	PL	Gr	Gr	PL	PL
Mass (pre), kg DM/ha	3957	3757	5018	3895	5045	3822	4856	46.5	0.09	74.5	0.26
Mass (post), kg DM/ha	1594^{b}	1401^{a}	1408^{a}	1513^{ab}	1496^{b}	1511^{ab}	1628^{c}	36.1	0.05	79.3	0.001
Grazed mass, kg DM/ha	2363	2356	3610	2382	3550	2312	3228	30.4	0.47	79.3	0.052
Pasture height (pre), cm	12.9	12.2	21.7	12.7	21.8	12.4	20.8	0.33	0.09	0.79	0.26
Pasture height(post), cm	4.5^{b}	3.8^{a}	2.5^{a}	4.2^{ab}	3.0^{b}	4.2^{ab}	3.7^{c}	0.256	0.05	0.17	0.001
Grazed height, cm	8.4	8.4	19.2	8.5	18.8	8.2	17.1	0.216	0.47	79.3	0.052

PL0= ryegrass-white clover only, PL15= 15% plantain & 85% ryegrass-white clover, PL30= 30% plantain & 70% ryegrass-white clover, PL60= 60% plantain & 40 ryegrass-white clover

SEM= Standard error of the means

$^{a\,b\,c}$ means in the same row with different superscripts differ significantly (P<0.05)

4.3 Botanical composition

Percentage botanical composition of perennial ryegrass-white clover and plantain on dry matter basis pre- and post-grazing is shown in Table 4.2.

4.3.1 Pre-grazing composition

The composition of perennial ryegrass, white clover and dead material in the perennial ryegrass-white clover sward were similar (P=0.738) amongst treatments averaging 72.6%, 17% and 8.0% respectively. Dicotyledonous (mainly *Chenopodium album* L) and monocotyledonous weeds were 1.8% and 0.5% in pasture sward, respectively. The composition of plantain sward was similar (P=0.548) among the 4 treatments comprising of 89.55% plantain2.1% ryegrass, 5.5% dead material and 2.7% weeds.

4.3.2 Post-grazing composition

The composition of ryegrass, white clover and dead material in the perennial ryegrass-white clover sward were similar among treatments averaging 77.1%, 5.5% and 16.3% respectively. Dicotyledonous and monocotyledonous weeds were 0.7% and 0.5% in pasture sward, respectively. The composition of plantain sward was similar among treatments comprising of 86.8% plantain, 0.6% ryegrass, 10.3% dead material and 1.7% weeds.

Table 4.2 Pre and post-grazing botanical composition (%DM) of perennial ryegrass-white clover (Gr) and plantain (PL) offered at 25kg DM/cow/day to late lactation cows in autumn (*P*- value for comparison of pastures within treatments (Gr) and plantain (PL) within treatments are are shown). PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain

	PL0	PL15		PL30		PL60		SEM	P-value	SEM	P-value
	Gr	Gr	PL	Gr	PL	Gr	PL	Gr	Gr	PL	PL
				% composition in pasture and plantain pre-grazing							
Ryegrass	73.2	68.3	3.3	70.8	2.8	78.1	0.1	6.34	0.73	1.70	0.42
[1]WC	16.4	22.3	0.2	17.1	0.0	12.14	0.1	6.21	0.73	0.12	0.44
Plantain	0.1	0.2	87.8	0.0	86.5	0.0	94.3	0.13	0.64	5.03	0.55
[2]Dead	7.3	7.6	5.5	9.0	6.1	8.2	5.0	0.36	0.06	1.32	0.85
[3]Weed DC	2.2	1.4	0.1	2.9	4.5	0.7	0.2	1.58	0.79	2.53	0.45
[4]Weed MC	0.8	0.1	3.1	0.3	0.1	0.8	0.2	0.26	0.23	1.80	0.48
				% composition in pasture and plantain post-grazing							
Ryegrass	79.1	77.0	0.1	75.3	0.80	76.8	0.8	2.93	0.84	0.36	0.37
[4]WC	4.3	7.7	0.0	6.1	1.81	3.9	0.1	2.20	0.62	1.04	0.46
Plantain	0.0	0.0	90.5	0.1	80.6	0.0	89.4	0.05	0.46	4.01	0.27
Dead	15.4	14.8	9.0	17.3	12.2	17.5	9.5	1.60	0.56	2.47	0.64
Weed DC	0.8	0.5	0.1	1.1	4.40	0.3	0.0	0.51	0.76	2.32	0.40
Weed MC	0.4	0.0	0.31	0.0	0.20	1.5	0.13	0.70	0.49	0.12	0.60

[1]WC= white clover, [2]Dead= Dead material, [3]Weed DC= Dicotyledon weeds, [4]Weed MC= Monocotyledon weeds

4.3.3 Feed major nutrient chemical composition

The chemical composition of pasture and plantain pre and post grazing are shown in Table 4.3. All individual nutrients were similar in perennial ryegrass-white clover and plantain respectively across the treatments for both pre-grazing and post-grazing. Neutral detergent fibre (%) was different between pre-grazing and post-grazing pasture and plantain (42.4 *vs*. 24.7 and 47.2 *vs*. 26.6 respectively). At approximately 20.5% CP each, pre-grazing pasture and plantain had similar CP but the average was lower in post-grazing (18.2%) for both grasses. Metabolisable energy (MJ/kg DM) was slightly higher for plantain (12.8) than pasture (12.3) pre-grazing. Ash was similar for plantain and pasture (approximately 10%) pre-grazing, but higher in the post-grazing (12.4%).

Table 4.3 Chemical composition (% DM) and ME (MJ/kg DM) of ryegrass-white clover and plantain offered to late lactation cows in autumn (P- value for comparison of pastures within treatments (Gr) and plantain (PL) within treatments are shown). PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain. *Ash = 100%-Organic matter (OM) (CSIRO, 2007)

	Treatments										
	PL0	PL15		PL30		PL60		SEM	*P*-value	SEM	*P*-value
Nutrient	Gr	Gr	PL	Gr	PL	Gr	PL	Gr	Gr	PL	PL
				Pre-grazing							
ADF	24.2	23.5	17.5	24.0	17.8	23.7	18.3	0.34	0.55	0.23	0.16
WSC	16.8	17.1	16.8	16.8	17.1	17.0	16.1	0.29	0.82	0.34	0.22
DMD	79.3	79.5	83.5	79.3	83.5	79.7	83.9	0.15	0.27	0.08	0.04
DOMD	76.6	76.8	80.0	76.5	79.7	77.1	79.7	0.17	0.15	0.23	0.65
NDF	43.4	41.2	24.8	42.1	25.0	43.1	24.7	1.30	0.64	0.29	0.75
OM	90.6	90.6	89.9	90.6	89.7	90.6	89.3	0.16	0.99	0.2	0.26
OMD	85.3	85.4	89.0	85.2	88.8	85.6	88.9	0.17	0.43	0.13	0.72
CP	23.1	24.1	20.7	23.6	20.5	23.6	20.4	0.61	0.76	0.24	0.82
DM	93.5	93.5	92.5	93.5	92.7	93.6	93.5	0.12	0.88	0.18	0.03
ME	12.3	12.3	12.8	12.2	12.8	12.3	12.8	0.03	0.15	0.04	0.65
*Ash	9.5	9.4	10.1	9.5	10.3	9.4	10.7	0.16	0.99	0.20	0.26
					Post-grazing						
ADF	25.0	26.7	21.7	26.8	21.4	26.5	21.6	1.20	0.70	0.41	0.81
CHO	18.4	18.6	11.8	19.4	12.8	19.5	13.0	0.66	0.57	0.67	0.46
DMD	76.5	76.6	80.0	77.2	80.6	77.0	81.1	0.28	0.28	0.41	0.28
DOMD	72.2	73.0	71.5	73.2	73.2	72.8	73.8	0.52	0.38	1.07	0.38
NDF	48.8	46.1	26.8	47.2	26.4	46.8	26.8	0.87	0.27	0.29	0.59
OM	89.3	88.7	85.6	89.4	86.5	89.1	86.4	0.29	0.39	0.54	0.50
OMD	82.0	81.8	82.6	82.7	83.3	82.5	84.1	0.34	0.29	0.73	0.42
CP	18.2	18.9	18.4	18.7	19.0	18.4	18.1	0.49	0.76	0.54	0.53
DM	93.2	93.3	93.2	93.2	93.3	93.2	93.7	0.07	0.61	0.08	0.02
ME	11.6	11.5	11.4	11.7	11.7	11.7	11.8	0.12	0.38	0.17	0.39
*Ash	10.7	11.3	14.4	10.6	13.5	10.9	13.6	0.29	0.39	0.54	0.50

4.4 Mineral, bioactive compound feed composition and dietary cation-anion difference (DCAD)

The mineral composition for perennial ryegrass-white clover and plantain and bioactive glycoside compound composition of plantain are shown (Table 4.4).

4.4.1 Mineral concentration

Individual minerals were similar across the treatments for pre- and post-grazing plantain and perennial ryegrass-white clover. Plantain had higher S, Cl, and Na concentration than perennial ryegrass-white clover (0.27%, 2.63%, and 1.14% *vs.* 0.23%, 1.60% and 0.56% respectively) pre-grazing. Perennial ryegrass-white clover had higher K and Mg concentration than plantain (4.4% and 0.2% *vs*. 3.1% and 0.2% respectively) pre-grazing.

4.4.2 Plantain bio-active glycosides

The bioactive glycosides (catalpol, aucubin, acteoside) were each similar across the treatments pre- and post-grazing. Catalpol concentration in plantain was low, averaging 0.01 and 0.01 mg/g dry weight (DW) in pre- and post-grazing herbage respectively. The concentration aucubin concentration pre-grazing was 4.63 mg/g DW per treatment compared to 2.87 mg/g DW in post-grazing herbage. Acteoside concentration was lower in pre-grazing (2.29 mg/g DW) than in post-grazing herbage (2.79 mg/g DW).

Table 4.4 Mineral (%DM) and bioactive glycoside (mg/g dry DM) composition of perennial ryegrass-white clover (Gr) and plantain (PL) offered late lactation cows in autumn (P- value for comparison of pastures within treatments (Gr) and plantain (PL) within treatments are are shown; - Gr was not tested for bioactive compounds). PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain

	Treatment										
	PL0	PL15		PL30		PL60		SEM	*P*-value	SEM	*P*-value
Variate	Gr	Gr	PL	Gr	PL	Gr	PL	Gr	Gr	PL	PL
			Mineral composition (%) pre-grazing								
P	0.29	0.29	0.30	0.28	0.30	0.28	0.31	0.0062	0.650	0.014	0.790
K	4.62	4.15	2.80	4.38	3.03	4.40	3.47	0.196	0.476	0.197	0.164
S	0.24	0.23	0.26	0.23	0.27	0.23	0.28	0.0035	0.221	0.014	0.633
Ca	0.63	0.63	2.01	0.62	2.12	0.61	2.09	0.038	0.989	0.145	0.864
Mg	0.22	0.22	0.14	0.22	0.15	0.22	0.15	0.004	0.913	0.010	0.967
Na	0.52	0.58	1.27	0.57	1.15	0.58	0.99	0.062	0.873	0.135	0.409
Cl	1.68	1.51	2.47	1.62	2.53	1.57	2.90	0.060	0.349	0.308	0.336
			Mineral composition (%) post-grazing								
P	0.28	0.28	0.30	0.26	0.33	0.29	0.30	0.008	0.264	0.012	0.228
K	4.80	4.30	3.10	4.43	3.10	4.50	3.43	0.202	0.421	0.287	0.667
S	0.22	0.23	0.22	0.21	0.23	0.21	0.23	0.010	0.522	0.004	0.790
Ca	0.57	0.60	2.02	0.62	1.91	0.60	2.05	0.0360	0.970	0.044	0.170
Mg	0.22	0.22	0.20	0.22	0.18	0.22	0.18	0.007	0.870	0.005	0.129
Na	0.52	0.57	1.18	0.60	1.20	0.59	1.23	0.030	0.037	0.112	0.958
Cl	1.75	1.60	1.90	1.68	1.75	1.62	3.03	0.0939	0.697	0.382	0.138
			Bioactive glycosides composition (mg/g DW) pre-grazing								
Catalpol	-	-	0.016		0.009		0.008	-	-	0.005	0.493
Aucubin	-	-	4.77		4.27		4.86	-	-	0.494	0.685
Acteoside	-	-	2.30		2.50		2.06	-	-	0.432	0.784
			Biocative glycoside composition (mg/g DW) post-grazing								
Catalpol	-	-	0.008		0.011		0.009	-	-	0.002	0.404
Aucubin	-	-	2.42		2.88		3.31	-	-	0.384	0.364
Acteoside	-	-	3.08		1.88		3.46	-	-	0.552	0.229

4.5 Animal performance

4.5.1 Feed dry matter intake

The dry matter intake (DMI) of feed and feed components is shown in Table 4.5. The cows in PL15, PL0, and PL30 consumed similar total DM (15.98 kg DM/cow/day), 1.23 kg above DMI of cows grazing ryegrass-white clover (*P*=0.003). Concentrations of the nutrient intake are also presented in Table 4.7. Most of the nutrient components' intake concentration did not respond to treatment change. Nutrient detergent fibre (g/kg DM) declined from 398.8 in PL0 to 298.3 in PL60 (*P*=0.012). Dry matter digestibility (DMD) increased with plantain proportion (PL0=PL15 and PL30<PL60) (*P*=0.004). The concentration of Ca eaten was higher for PL60 than PL0 (16.25 *vs*. 6.68 g/kg DM). Magnesium consumed in the PL60 treatment was also highly concentrated than in PL0 by 78.58% (*P*<0.001).

4.5.2 Nutrient in diet and feed selection

Nutrient concentration of feed offered was estimated (Table 4.6). As the plantain proportion increased in the diet, the fibre intake decreased. Neutral detergent fibre (NDF) declined from PL0 to PL60, with PL60, having 28.6% less NDF than PL0, whilst PL15 and PL30 had the same NDF (370.3 g/kg DM (*P*<0.001). Metabolisable energy was highest for treatment PL60 (12.6 ME/kg DM) by 2.85% compared with PL0 (12.3 ME/kg DM), whilst pasture in PL15 and PL30 had similar ME (12.4 ME/kg DM). Digestibility of dry matter (DMD) was greatest (824.4 g/kg DM) compared with 793.4, 802.7 and 807.8 g/kg DM for PL0, PL15 and PL30 respectively (*P*<0.001). Digestibility of organic matter (DOMD) increased (*P*<0.001) with the inclusion of more plantain in the diet with PL60 having the highest digestibility (788.1 g/kg DM compared with 765.5, 774.3 and 776.5 g/kg DM in PL0, PL15 and PL30 respectively). Crude protein, OM and WSC were all similar among treatments *(P*=0.08, *P*=0.08 and P=0.43, respectively).

Less crude protein (CP) was selected in the offered diet as the plantain proportion increased in the diet, declining from PL0 to PL60 by 7.89%. Cows selected less ME (*P*=0.005) with an increase of plantain in the diet (e.g. 1.039 vs. 1.012 for PL0 and PL60 respectively. Selection of more digestible material increased (*P*=0.005) with the decline of plantain in the diet from treatment PL60 to PL0.

Minerals in the offered diet were estimated (Table 4.6). Potassium concentration in PL60 treatment was 9.15% lower than the average (41.3 g/kg DM) of the other 3 treatments that were similar to each other (*P*=0.019). Calcium increased (*P*<0.001) with plantain proportion increasing in the offered diet from 6.28 g/kg DM in PL0 to 15.8 g/kg in PL60, with PL0 & PL15 (7.6 g/kg DM) and PL30 & PL60 (13.7 g/kg DM) having the similar Ca respectively. Chloride concentration in the offered diet was 27.7% higher than the average (17.7 g/kg DM) of PL0, PL15 and PL30 that were similar to each other (*P*<0.001). As plantain increased in the diet, so did Mg (P<0.001). This mineral had the highest concentration in PL60 (7.3 g/kg DM) than the rest of the treatments that had an average of 3.6 g/kg DM). Sulphur concentration in the offered diet was similar in PL30 and PL60 (2.5 g/kg DM) but different from that in PL0 and PL15 (2.3 g/kg DM) that was similar. The concentration of Na and P did not change with the increased proportion of plantain in the diet.

The cows selected herbage with more Mg as the plantain increased in the diet (1.01 to 1.40 g/kg DM from PL0 to PL60 respectively ($P<0.001$). In contrast, the cows consumed less Cl and K than that offered in the diet (selection differential of 0.99 and 0.93 respectively). Phosphorus, S, Ca and Na were each selected similarly selected amongst treatments (1.02, 1.07, 1.05 and 1.01 respectively).

Table 4.5 Dry matter and nutrient intake of perennial ryegrass-white clover and plantain offered in different proportions to late lactating cows in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

	Treatment						Treatment					
	PL0	PL15	PL30	PL60	SEM	*P*-value	PL0	PL15	PL30	PL60	SEM	*P*-value
DMI (kg) and nutrient intake (g/cow/day) and ME intake (MJ/kg DM/cow/day)							Concentration of nutrient intake (g/kg DM) and ME intake (MJ/kg DM) /cow /day					
DMI (kg)	14.74^{a}	15.96^{b}	16.00^{b}	15.97^{b}	0.160	0.003						
ADF	3494.3	3315.1	3277.8	3102.1	141.60	0.360	237.24	207.61	204.87	194.22	8.920	0.062
WSC	2318.8	2612.7	2531.7	2531.0	76.50	0.138	157.36	163.77	158.20	158.42	5.400	0.829
DMD	11977.7^{a}	13051.2^{b}	13074.5^{b}	13271.9^{b}	117.10	<.001	812.74^{a}	817.60^{a}	817.06^{a}	830.97^{b}	2.080	0.004
DOMD	11716.2^{a}	12740.3^{b}	12646.7^{b}	12740.9^{b}	91.40	<.001	795.13	798.19	790.32	797.75	4.000	0.531
NDF	5872.6	5659.7	5357.2	4764.0	237.30	0.066	398.83^{b}	354.56^{ab}	334.84^{ab}	298.27^{a}	13.940	0.012
OM	13472.0^{a}	14574.4^{b}	14495.8^{b}	14379.2^{b}	132.80	0.003	914.22	913.04	905.91	900.35	3.240	0.069
OMD	12904.6	14025.7	14010.2	14129.8	118.40	<.001	875.68	878.70	875.52	884.70	3.150	0.238
CP	3897.6	4111.2	3882.2	3601.7	119.40	0.113	264.46	257.57	242.60	225.59	7.960	0.051
N	623.6	657.8	621.2	576.3	119.40	0.113	42.52	41.21	38.81	36.09	7.960	0.051
ME	187.5^{a}	203.9^{b}	202.4^{b}	203.9^{b}	1.46	<.001	12.72	12.77	12.65	12.76	0.064	0.531
Ash	1265.8^{a}	1389.3^{ab}	1505.7^{ab}	1592.1^{b}	57.40	0.030	85.78	86.96	94.09	99.65	3.240	0.069
P	44.6	47.1	47.2	48.1	1.48	0.456	3.03	2.96	2.95	3.01	0.114	0.939
K	563.8	588.7	609.4	597.4	44.80	0.902	38.19	36.89	38.08	37.42	3.09	0.989
S	37.4a	37.2a	42.4^{ab}	46.6^{b}	1.56	0.015	2.54^{ab}	2.33^{a}	2.65^{ab}	2.92^{b}	0.084	0.014
Ca	98.6^{a}	150.1^{ab}	197.9^{bc}	259.9^{c}	18.06	0.004	6.68^{a}	9.41^{a}	12.36^{ab}	16.25^{b}	1.059	0.003
Mg	32.5^{a}	71.5^{b}	105.8^{c}	164.0^{d}	1.323	<.001	2.20^{a}	4.48^{b}	6.61^{c}	10.27^{d}	0.050	<.001
Na	88.4	117.7	122.8	122.4	21.2	0.635	6.03	7.33	7.67	7.66	1.321	0.793
Cl	231.2^{a}	264.0^{ab}	330.8^{ab}	398.1^{b}	28.2	0.023	15.65^{a}	16.47^{ab}	20.67^{ab}	24.89^{b}	1.611	0.022

$^{a\ b\ c}$ means in the same row with different superscripts differ significantly (P<0.05)

Table 4.6 Nutrient concentration and selection differentials of ryegrass-white clover and plantain offered at different proportions to late lactating cows in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

Nutrient	Treatment PL0	PL15	PL30	PL60	SEM	*P*-value	Treatment PL0	PL15	PL30	PL60	SEM	*P*-value
	Concentration of nutrient offered (g/kg DM)						Selection differential of nutrient offered					
ADF	241.9^{c}	223.7^{b}	217.6^{b}	201.4^{a}	1.83	<.001	1.0	0.9	0.9	1.0	0.04	0.72
WSC	168.1	170.4	168.6	163.9	2.69	0.43	0.9	1.0	0.9	1.0	0.02	0.64
DMD	793.4^{a}	802.7^{b}	807.8^{b}	824.4^{c}	1.32	<.001	1.0^{c}	1.0^{bc}	1.0^{ab}	1.0^{a}	0.002	0.002
DOMD	765.5^{a}	774.3^{ab}	776.5^{b}	788.1^{c}	1.76	<.001	1.0^{c}	$1.0b^{c}$	1.0^{ab}	1.0^{a}	0.003	0.005
NDF	434.4^{c}	380.8^{b}	359.9^{b}	310.0^{a}	7.99	<.001	0.9	0.9	0.9	1.0	0.02	0.48
OM	905.5	904.3	902.0	897.5	1.80	0.08	1.0	1.0	1.0	1.0	0.003	0.095
OMD	853.0^{a}	860.7^{ab}	864.7^{b}	877.4^{c}	1.59	<.001	1.0^{b}	1.0^{ab}	1.0^{ab}	1.0^{a}	0.02	0.02
CP	231.1	234.1	224.7	215.2	4.35	0.08	1.1^{c}	1.1^{bc}	1.1^{ab}	1.1^{a}	0.007	0.004
ME	12.3^{a}	12.4^{ab}	12.4^{b}	12.6^{c}	0.03	<.001	1.0^{c}	1.0^{bc}	1.0^{ab}	1.0^{a}	0.003	0.005
Ash	94.5	95.7	98.0	102.5	6.22	0.08	0.9	0.9	1.0	1.0	0.02	0.10
P	2.9	2.9	2.9	3.00	0.07	0.54	1.0	1.0	1.0	1.0	0.02	0.71
K	46.2^{b}	38.9^{ab}	39.0^{ab}	37.9^{a}	1.40	0.02	0.8	1.0	1.0	1.0	0.05	0.25
S	0.24^{ab}	0.23^{a}	0.24^{ab}	0.26^{b}	0.01	0.027	1.1	1.0	1.1	1.1	0.03	0.25
Ca	6.3^{a}	8.9^{ab}	11.5^{bc}	15.8^{c}	0.78	<.001	1.1	1.1	1.1	1.0	0.02	0.60
Mg	2.2^{a}	3.7^{b}	5.0^{c}	7.3^{d}	0.04	<.001	1.0^{a}	1.2^{b}	1.3^{c}	1.4^{c}	0.02	<.001
Na	5.2a	7.2a	7.8	8.5	0.95	0.06	1.1	1.0	1.0	0.9	0.10	0.48
Cl	16.8^{a}	16.9^{a}	19.5^{a}	24.4^{b}	0.67	<.001	0.9	1.0	1.1	1.0	0.06	0.52

$^{a\ b\ c}$ means in the same row with different superscripts differ significantly (P<0.05).

4.5.3 Dietatry cation-anion difference (DCAD)

The dietary cation-anion difference (DCAD) estimated from the minerals in the offered diet is shown in Table 4.5. The perennial ryegrass-white clover and plantain diet in PL60 had 34% less DCAD than the average DCAD in PL0, PL15 and PL30 that were similar to each other (*P*<0.002). DCAD could have declined in PL60 beacuse of the difference between the cations (Na + K) and anions (Cl + S). Potassium in PL60 was 8.5% less than the average of PL0, PL15 and PL30 (*P*=0.019). For the anions, Cl in PL60 was 27.45% higher than the average of Cl in PL0, PL15 and PL30 (*P*<0.001). Phosphorus and Na consumed in the diet (Table 4.5) were similar between the treatments.

Table 4.7 Minerals (%) in the offered diet and estimated DCAD (mEq/kg DM) of perennial ryegrass-white clover and plantain in different proportions grazed by late lactating cows in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

	Treatment					
Mineral	PL0	PL15	PL30	PL60	SEM	*P*-value
P	0.29	0.29	0.29	0.30	0.0065	0.542
K	4.62^{b}	3.89^{ab}	3.90^{ab}	3.79^{a}	0.1395	0.019
S	0.24^{ab}	0.23^{a}	0.24^{ab}	0.26^{b}	0.0057	0.027
Ca	0.63^{ab}	0.89^{ab}	1.15^{ab}	1.58^{b}	0.0782	<.001
Mg	0.22^{a}	0.37^{b}	0.50^{c}	0.73^{d}	0.0035	<.001
Na	0.52	0.71	0.78	0.85	0.067	0.058
Cl	1.68^{a}	1.69^{a}	1.95^{a}	2.44^{b}	0.067	<.001
*DCAD	787.0^{b}	683.2^{b}	636.4^{ab}	483.9^{a}	28.3	0.002

$^{a\ b\ c}$ means in the same row with different superscripts differ significantly (*P*<0.05)
*Dietary cation-anion difference (DCAD) was estimated using the equation: DCAD (mEq/kg DM) = [(%Na x 434.98) + (%K x255.74)] - [(%Cl x 282.06) + (%S x 623.75)] (Lean & DeGaris, 2010).

4.5.4 Water Intake

Drinking water intake was significantly different (*P*=0.002) among the treatments with a decline as the plantain proportions increased with PL0, PL15 similar (34.8 litres) and greater than PL60 (21.7 litres). Intake of water from feed averaged 109.9 litres/cow and increased from PL0 to PL60. The greatest total water intake was between PL0 (132.3 L) and PL60 (139.6 L) with PL15, PL30 and PL60 having a similar total water intake (*P*=0.035).

Table 4.8 Water intake of cows grazing spatially separated plantain and ryegrass/white clover in different proportions in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

Item	Treatments				SEM	*P*-value
	PL0	PL15	PL30	PL60		
Drinking water intake (L/cow)	38.2^{b}	31.4^{b}	29.3^{ab}	21.7^{a}	1.64	0.002
Feed water intake (L/cow)	94.2^{a}	108.1^{b}	119.3^{b}	117.9^{b}	2.06	<.001
Total water intake (L/cow/day)	132.3^{a}	139.5^{ab}	148.6^{ab}	139.6^{b}	2.79	0.035

$^{a\ b\ c}$ means in the same row with different superscripts differ significantly (P<0.05)

4.5.5 Milk yield and composition

There was no significant effect of plantain proportions on milk production parameters (Table 4.8) with milk yield and milk solids averaging 16.1 L and 1.60 L/cow/ day respectively. There was a slight rise of milk protein (kg /day) from 0.68kg to 0.72kg from PL0 to PL60 respectively, but the increase was not significant (P=0.937) among the treatments. Consequently, the feed conversion efficiency of milk solids (FCE_{ms}) and milk yield (FCE_{my}) were not different between treatments (P=0.231 and P=0.751 respectively). Body condition score was not different among the treatments, with an average gain of 0.025/cow/day resulting in a BCS of 4.2 at the end of the trial. The liveweight at the end of the experiment was similar among the four treatments (P=0.143). The average liveweight gain/ cow/day was 0.17 kg, resulting in an average weight of 510 kg/cow at the end of the experiment.

Table 4.9 Milk yield (kg/day/cow) and composition (kg and %/day/cow), liveweight (kg/day/cow), FCE and BCS changes of late lactating cows grazing ryegrass/white clover and plantain in different spatial proportions in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

	Treatment					
Component	PL0	PL15	PL30	PL60	SEM	*P-value*
Milk yield (kg)	15.8	16.1	16.4	16.1	0.712	0.937
Fat (kg)	0.89	0.90	0.91	0.89	0.030	0.986
Protein (kg)	0.68	0.71	0.72	0.72	0.015	0.380
Milk-solids (kg)	1.57	1.61	1.62	1.61	0.041	0.866
Fat (%)	5.77	5.81	5.79	5.75	0.240	0.993
Protein (%)	4.42	4.53	4.48	4.63	0.106	0.532
Prot :Fat	0.80	0.80	0.81	0.83	0.021	0.774
*FCE_{ms}	0.11	0.10	0.10	0.10	0.002	0.231
*FCE_{my}	1.08	1.01	1.03	1.01	0.050	0.751
Liveweight, start (kg)	480	486	505	502	7.88	0.171
Liveweight, final (kg)	498	495	525	522	5.61	0.143
Liveweight gains (kg)	1.81	0.83	1.98	2.02	0.304	0.096
BCS, start	3.88	3.96	3.96	3.96	0.098	0.782
BCS, final	4.08	4.21	4.21	4.25	0.075	0.485
BCS gain (kg/cow/day)	0.02	0.03	0.03	0.03	0.016	0.965

*FCE_{ms}= Feed conversion efficiency of milk solids
*FCE_{my}= Feed conversion efficiency of milk yield

4.5.6 Nitrogen partitioning

The results of nitrogen partitioning in milk, blood plasma, urine and faeces are presented in Table 4.10. Milk urea and milk urea nitrogen (MUN) decreased as plantain proportion increased in the diet (*P*=0.004). Compared with PL0, PL60 had the highest reduction (18%) of N for both MUN and milk urea. Nitrogen excretion in milk (g/day) was similar among treatments (*P*=0.605).

In blood plasma, creatinine, urea N and urea (mmol/L) were each unaffected by the proportion of plantain among the treatments. There was a trend of N reduction from PL0 to PL60 for urea N and urea (mmol/L) (22.70 *vs*. 17.67 and 11.35 *vs*. 8.83, respectively).

There was a significant difference (*P*=0.012) in urine nitrogen concentration with lower concentration at 60% than 15%, 30% and 0% plantainin the diet, with PL60 having 33% less N than the control treatment. A similar trend (*P*=0.010) was observed for urea among the treatments. There was no difference among treatments for allontoin (*P*=0.38) and creatinine (*P*=0.189). Ammonia (NH_3) was greater at 60% plantain proportion than 15 and 30% but similar with 0% plantain (*P*=0.011). Hippuric acid concentration in the urine was reduced as the proportion of plantain in the diet increased (*P*=0.011) with the highest reduction in PL60 compared with PL0 (6.18 *vs*. 3.98 mmol/L). The largest difference in N output (g/day) (*P*<.001) in urine was between PL0 and PL60, with PL60 output 20.9%

lower than that of PL0. Considering the N intake (g/day/cow) in Table 4.5 and comparing it with the N output (g/day), N retained increased with the inclusion with plantain. The greatest N rentention (25.2%) was in PL60 and this was 49.8% more than that in PL0. Urine pH was not different (*P*=0.258) among treatments with an average of 7.85 per treatment. The faeces DM and N percentage were similar among the 4 treatments with an average of 93.6 and 3.77 per treatment, respectively.

Table 4.10 Nitrogen partitioning in milk, blood plasma, urine and faeces of late lactation cows grazing spatial proportions of perennial ryegrass-white clover and plantain pastures offered in different proportions in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

	Treatments					
Variate	PL0	PL15	PL30	PL60	SEM	*P- value*
Milk						
Urea (mmol/L)	8.12^{c}	7.64^{bc}	7.29^{ab}	6.62^{a}	0.197	0.004
MUN (mmol/L)	16.2^{c}	15.3^{bc}	14.6^{ab}	13.2^{a}	0.395	0.004
N excretion (g/day)	109.7	114.0	114.5	116.9	3.67	0.605
Blood plasma						
Creatinine (mmol/L)	0.07	0.08	0.07	0.07	0.002	0.354
Urea N (mmol/L)	22.7	21.1	20.6	17.7	1.078	0.077
Urea (mmol/L)	11.4	10.6	10.3	8.83	0.539	0.077
Urine						
N (%)	0.45^{b}	0.41^{ab}	0.40^{ab}	0.30^{a}	0.023	0.012
Urea (mmol/L)	141.6^{b}	131.7^{ab}	127.8^{ab}	97.5^{a}	6.94	0.010
Allontoin	4.53	4.56	4.42	3.87	0.299	0.380
Creatinine (mmol/L)	0.78	0.84	0.82	0.70	0.045	0.189
NH_3 (mmol/L)	0.76^{ab}	0.56^{a}	0.55^{a}	0.85^{b}	0.048	0.011
Hippuric acid	6.18^{b}	5.30^{ab}	4.91^{ab}	3.98^{a}	0.336	0.011
Uric acid	0.55	0.55	0.49	0.40	0.049	0.189
N output (g/day)	545.4^{c}	459.1^{ab}	482.6^{b}	431.3^{a}	8.560	<.001
Urine pH	7.90	7.86	8.05	7.60	0.141	0.258
Faeces						
DM (%)	95.7	91.8	94.8	92.1	2.430	0.604
Ash (%)	22.8	23.0	23.0	23.3	0.485	0.931
N DM (%)	3.70	3.68	3.86	3.85	0.084	0.356

$^{a\ b\ c}$ means in the same row with different superscripts differ significantly (*P*<0.05)

4.5.7 Purine derivatives and microbial N production

The estimations of microbial N were based on purine derivatives and metabolic weight (Table 4.11). Microbial N supply an indication of microbial activity and total PD supply were similar among the treatments (*P*=0.268 and *P*=0.393, respectively).

Table 4.11 Purine derivatives and microbial N production in cows grazing perennial ryegrass-white clover and plantain grown in different spatial strip monocultures in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

Variates	Treatment					
Purine derivatives	PL0	PL15	PL30	PL60	SEM	*P*-value
Allantoin	4.53	4.56	4.42	3.87	0.311	0.425
Uric acid	0.55	0.55	0.49	0.40	0.056	0.290
[1]Total PD	5.08	5.10	4.90	4.27	0.360	0.393
Creatinine	0.784	0.84	0.82	0.70	0.050	0.283
Allantoin: creatinine ratio	5.79	5.42	5.36	5.53	0.128	0.192
kg $W^{0.75}$	103.3	104.2	107.1	107.5	0.861	0.032
[2]PD index	670.0	631.0	637.0	655.3	13.860	0.277
[3]Microbial N (g N/day)	410.1	384.3	387.5	399.3	9.070	0.268

[1]Total PD= allontoin + uric acid

[2]PD index = [(total PD)/creatinine] x $BW^{0.75}$

Microbial N was determined assuming daily purine derivative excretion (dPD; mmol/kg $BW^{0.75}$= PD index × 0.9

Daily absorbed purines (daP)= [dPD(mmol/kg of $BW^{0.75}$)- 0.385 × $BW^{0.75}$ + 0.85

[3]Microbial N (g N/day)= daP ×70)/(0.116 ×0.83 × 1000).

4.6 Animal behaviour

4.6.1 Grazing time and preference

A summary of time allocation to activities (grazing, ruminating and idling) and bite rate of the cows/treatment is shown in Table 4.11. Time allocated to grazing pasture differed (*P*=0.006 for the morning grazing, *P*=0.020 in the afternoon grazing), declining as the plantain proportion increased in both cases. In contrast, the time allocated to grazing plantain sward was similar in the morning grazing and the afternoon one among the treatments with plantain. On average, when comparing treatments with perennial ryegrass-white clover and plantain (PL15, PL30 and PL60), the cows spent more time grazing grass than plantain in the afternoon (62.08 *vs*. 34.86 minutes/cow respectively). In the PL60 treatment the cows spent more time on plantain than grass (55.00 vs. 40.63 minutes/cow respectively). In the morning grazing, each cow spent more time grazing plantain than pasture (47.3 *vs*. 43.5 minutes respectively). Preference of grazing between the two pastures was not affected by the treatments in the morning or afternoon grazing bouts. On average, cows preferred plantain in the morning, spending 52.1% of their grazing time on it compared with 47.9% on perennial ryegrass-white clover. However, in the afternoon grazing bout, 65.6% of the time was spent grazing grass than plantain (34.4%).

The cows spent similar time ruminating across the treatments in the morning and afternoon grazing (*P*=0.500 and *P*=0.298 respectively). Despite no differences, time spent ruminating declined with more proportion of plantain in the diet (e.g. in the afternoon grazing each cow spent 20 minutes ruminating in PL0 *vs*. 10 minutes ruminating in PL60). Time spent idling was distinct in the morning increasing from 10.8 minutes in PL0 to 46.4 minutes in PL60.

4.6.2 Bite rate

The average bite rate for cows grazing plantain and pasture was similar across treatments for the morning and afternoon grazing respectively (Table 4.12). On average the bite rate was higher (46 bites/minute/cow) when grazing perennial ryegrass-white clover than when grazing plantain (39 bites/minute/cow) in the morning grazing session but the difference was small in the afternoon (49 *vs*. 44 bites/minute/cow).

Table 4.12 Time allocation to activities and bite rate of cows grazing spatially separated perennial ryegrass-white clover and plantain offered in different proportions in autumn (PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain)

Variate	PL0	PL15	PL30	PL60	SEM	*P*-value	PL0	PL15	PL30	PL60	SEM	*P*-value
Time allocation (mins/120mins) to activities in the *morning							Time allocation (mins/120mins) to activities in the **afternoon					
Grazing pasture	105.6[b]	60.6[ab]	45.0[a]	25.0[a]	5.41	0.006	88.75[b]	68.13[ab]	77.50[ab]	40.63[a]	4.87	0.020
Grazing plantain	-	38.8	59.4	43.8	8.49	0.38	-	18.57	30.00	55.00	5.04	0.071
Total grazing during session	-	99.4	104.4	68.8	3.66	0.04	-	84.37	107.50	95.63	4.69	0.141
% time grazing grass		59.6	43.8	40.3	6.85	0.31		81.10	73.13	42.64	3.52	0.029
% time grazing plantain		40.4	56.2	59.7	6.85	0.306		18.90	26.90	57.4	3.52	0.029
Ruminating	16.67	11.25	5.00	21.25	9.49	0.500	20.00	21.67	15.00	10.00	4.56	0.298
Idling	10.83	20.00	21.00	46.43	3.96	0.029	15.71	22.14	13.75	18.13	4.10	0.539
Bite rate (bites/min) in the *morning grazing							Bite rate (bites/mins) in the **afternoon grazing					
Bites on pasture	47.4	48.0	47.6	40.4	4.20	0.587	47.4	50.6	49.5	47.2	3.55	0.875
Bites on plantain	-	37.9	38.5	37.9	7.36	0.998	-	45.1	46.8	38.7	5.27	0.605

[a b c] means in the same row with different superscripts differ significantly (P<0.05)

*morning grazing = 2hours after morning milking

**afternoon grazing = 2hours after afternoon milking

4.6.3 Urination and urine patch area

Urine patch area was calculated through two methods. Direct measurements from the infra-red photos of the urine patches, gave patch areas of 0.18 to 0.24m^2 for plantain and pasture and these were similar among the treatments (*P*=0.29). Figure 4.2 shows the relationship of patch area and volume of artificial urine applied. The curve was used for calibrating the urine patch size. Estimates from the calibration curve between the volume and area gave values ranging from 0.28 to 0.37m^2 (Table 4.13).

Volume per urination and total urination increased numerically up to PL30 and declined in PL60, with no difference amongst treatments (*P*=0.297 and 0.523 respectively). The number of urinations within 24hrs ranged from 9 and 13 times (Table 4.13).

Table 4.13. Urination and urine patch area in paddocks of cows grazing spatially separated perennial ryegrass-white clover and plantain pastures offered in different proportions(mean ± sem). PL0=0% plantain, PL15=15% plantain, PL30= 30% plantain, PL60= 60% plantain

	Treatments				
Variate	PL0	PL15	PL30	PL60	*P*-value
Direct urine patch area (m^2)	0.24 ± 0.033	0.26 ± 0.025	0.24 ± 0.033	0.18 ± 0.033	0.297
Calibrated urine patch area (m^2)	0.35 ± 0.033	0.37 ± 0.025	0.34 ± 0.033	0.28 ± 0.033	0.297
Urination frequency (times/24hrs)	9.0 ± 1.500	9.8 ± 1.162	13.3 ± 1.500	12.0 ± 1.500	0.195
Urination volume (ml/kg LWT)	5.5 ± 0.632	5.5 ± 0.489	4.5 ± 0.632	3.65 ± 0.632	0.160
Volume per urination (L)	2.46 ± 0.349	2.66 ± 0.270	2.4 ± 0.349	1.77 ± 0.349	0.297
Total urine volume (L/24hrs)	22.41 ± 4.948	25.5 ± 3.833	31.8 ± 4.948	22.5 ± 4.948	0.523

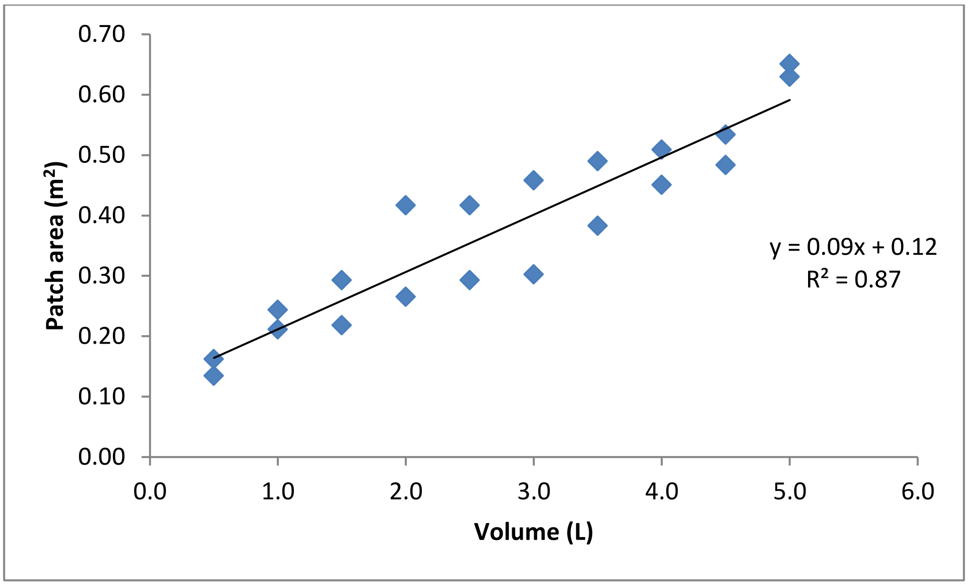

Figure 4.2. Calibration curve showing the relationship of artificially applied urine and the area of the urine patch (m^2).

Chapter 5 Discussion

The study was designed to test the effect of plantain proportions in the diet on milk production, N utilisation and behaviour of late lactating cows in pasture based systems. This was accomplished by comparing cows grazing strips of spatially separated monocultures of perennial ryegrass-white clover and plantain with strip width altered to change the proportions. The response variables were animal parameters (dry matter intake, milk, blood, urine, faeces, behaviour) and feed characteisristics (dry matter, botanical and feed composition).

5.1 Herbage characteristics

5.1.1 Herbage mass

Herbage mass offered pre-grazing was different between plantain (4973 kg DM/ha) and perennial ryegrass-white clover (3858kg DM/ha). This reflected differential growth rates in establishment of the experiment. Pastures were grazed 4 weeks prior to experiment but different growth rates meant that herbage mass pre-grazing was not even. This could explain the higher plantain yield than pasture with plantain-based pastures producing more (1.8 t DM/ha) and (0.9 t DM/ha) than ryegrass-white clover pastures in summer and in autumn respectively (Moorhead & Piggot, 2009). However Box *et al*. (2016) estimated similar pre-grazing herbage mass for pasture (3209kg/ha DM) and plantain (3187 kg/ha DM) in a trial done in autumn. The differences could be different stages and height at which the herbage was grazed.

5.1.2 Chemical composition

The chemical composition of pre-grazing pasture and plantain was not affected by weeds and dead material because individual spatial strips comprised >70%, weeds <3% and dead material <9%. Perennial ryegrass-white clover pre-grazing on average had higher CP (23.6% *vs*. 20.5), higher NDF (42.5% *vs*. 24.8%), lower ME (12.3 MJ/kg DM *vs*. 12.8 MJ/kg DM) than plantain herbage at pre-grazing. These results are consistant with the summary by Stewart (1996), that plantain has lower proportion of cell wall, less cellulose, less neutral and acid detergent fibre and less crude protein. In another study Navarrete *et al*. (2016) reported lower OM (85.6% *vs*. 89.6%), ME (12.2 *vs*. 12.8 ME/kg DM), NDF (18.9% *vs*. 24.8%) but higher values of CP (29.9% *vs*. 20.5%) for plantain. Crude protein of plantain (20.5%) was lower than 27% of 150mm high plantain reported by Lee, Hemmingson, Minnee, and Clark (2015) in autumn. Van Eekeren, Wagenaar, and Jansonius (2006) reported a lower CP of plantain (13.6%) and ryegrass-white clover (14.1%) over a study carried out over 2 years that was lower than in this study. The differences in CP could be that pasture in the latter study was 2 years old whereas in the current study the pasture was only 4 months old confirming that quality declines as plants lignify. Harrington *et al*. (2006) reported CP for pasture (23.2%) and plantain (28.3%) in a summer (January) pasture. Plantain ash concentration (10.4%) in this study was lower that that observed in other studies (Gregorini *et al*., 2013; Harrington *et al*., 2006; Lee *et al*., 2015; Navarrete *et al*., 2016; Van Eekeren *et al*., 2006) that ranged from 11.3-14.3%. The difference between the ash reported in the current and the latter ones could be attributed to varying age at sample collection stages, with new pasture in this study. The higher ash content (12.4%) in the post grazing plantain and ryegrass-white clover was attributed to soil contamination when the samples where collected at ground level.

5.1.3 Minerals, DCAD and bioactive compounds in plantain

Plantain had a higer concentration of minerals than pasture in pre-grazing herbage (1.14% vs 0.56, 2.07 vs 0.62%, 0.27 and 0.27 vs. 0.23% for Na, Ca, S respectively). This is consistent with findings by Van Eekeren *et al*. (2006) who reported 1.32 % vs 0.57% and 0.40 vs. 0.37 for Ca and S respectively, but a higher Na level in ryegrass (0.25%) than plantain (0.10%). In pre-grazing pasture, K and Mg concentration were higher (4.38%) in perennial ryegrass-white clover than in plantain (3.10%). The mineral concentration of plantain and perennial ryegrass-white clover reported elsewhere vary. Plantain Ca concentration in this study was higher (2.07%) than that reported in previous studies (average 1.55%) (Harrington *et al*., 2006; Van Eekeren *et al*., 2006). Sodium in plantain was also higher in the recent study than that reported by Van Eekeren *et al*. (2006) (1.14 *vs*. 0.10%) but lower than that presented by Harrington *et al*. (2006) (1.14 *vs*.3.37%). Phosphorus and K in plantain was similar to those reported in other studies (Harrington *et al*., 2006; Van Eekeren *et al*., 2006).

Dietary cation-anion difference (DCAD) (mEq/kg DM) estimated using the formula described by Lean and DeGaris (2010), declined with an increase in plantain proportions in the diet from 787.0 in PL0 to 483.9 in PL60. The decline of DCAD in this study could be caused by plantain that has low DCAD as reported in previous studies (Pembleton *et al*., 2015; Rugoho *et al*., 2016). In a diet comprising of rape and plantain, Rugoho *et al*. (2016), estimated a DCAD (mEq/kg DM) of 171 compared with 258 for perennial ryegrass.

Limited studies have analysed the bio-actives in plantain grazed by dairy animals. Catalpol (mg/g dry weight (DW)) was insignificant (0.008-0.010) in plantain herbage, similar to the findings by Navarrete *et al*. (2016). The aucubins and acteoside in plantain were 4.6 mg/g DW and 2.29 mg/g DW in the study which is within range of findings of Navarrete *et al*. (2016) (aucubins 1.78 to 3.80mg/g DW and acteaoside 0.5 to 41.7mg/g DW in the first season). Tamura and Nishibe (2002) found higher aucubin and acteoside in Tonic Ceres (10-27mg/g DW and 15-41mg/g DW respectively) than (4.63 and 2.29mg/g DW) in this study in the first season and that reported by Navarrete *et al*. (2016). The variations of glycosides reported in this study and that by Tamura and Nishibe (2002) Navarrete *et al*.

(2016) could be because of environmental differences of experimental sites, age of the pastures and sampling proceedures.

5.2 Animal performance

5.2.1 Selection of nutrients

Dry matter intake was established through nutrients offered and their selection differentials. Selection differentials of most nutrients (ADF, WSC, NDF, OM) were similar amongst the treatments with cows selecting nutrients ranging between 0.82- 1.14 times the offered in diet. Cows selected more CP in PL0 and PL15 (1.14 and 1.10 respectively) than other nutrients, with PL60 having a lower selection differential for CP. The average selection differential for CP were lower (1.09) than (1.32) reported earlier in perennial ryegrass (Moate, Dalley, Roche, & Grainger, 1999; Wales, Doyle, Stockdale, & Dellow, 1999). This may have been due to the difference in age with the pasture in this study being younger (4 months). On average the nutrient selection differentials in this study were in the range of those reported in ryegrass in earlier reports (Moate *et al*., 1999; Wales *et al*., 1999).

Cows selected minerals similarly across treatments; except for Mg that was differently selected. For this mineral, the cows selected more with the increase of plantain in the diet (1.01 *vs*. 1.40 for PL0 and PL60 respectively). The PL0 selection differentials for Mg are within the range of that in earlier studies (Moate *et al*., 1999; Wales *et al*., 1999). The reasons for difference are unclear.

5.2.2 Feed dry matter intake

Daily herbage dry matter intake (kg DM/cow) estimated from disappearance of herbage pre- and post- grazing was lower for PL0 (15.0) than PL15, PL30 and PL60 (16.0). Edwards *et al*. (2015) reported similar intakes (16.2kg) for diverse pastures with plantain and (15.3kg) for perennial ryegrass-white clover for mid lactating cows in summer. The results in this study were, however higher than those found by Totty *et al*. (2013) that were similar for diverse pastures, high sugar perennial ryegrass-white clover and ryegrass-white clover (14.3kg) for late lactating cows in autumn. The apparent DMI for cows grazing 100 % pasture and 60% plantain in this experiment was higher (15.0kg vs. 14.0kg) and similar (16.0kg) to that reported by Box *et al*. (2016) during autumn for late lactating cows. The increase in plantain in DM may give the ease of harvest of the plants with lower post-grazing herbage mass for plantain than in pasture observed during the experiment (3.1 cm *vs*. 4.2 cm). However, caution is needed in interpreted results as they are calculated from pre- and post-grazing values not direct measurements. The herbage disappearance method has errors associated with it (Moore, 1996), but this method has been successfully used in previous studies in New Zealand (Totty *et al*., 2013). The concentration of ingested NDF could also explain the higher DMI in diets with plantain, because with the increase of plantain in the diet, the concentration of NDF declined. The concentration of feed ingested (g/kg DM) was not different for WSC (159.4), CP (247.6), ash (91.6), DOMD (795.3) and ME (MJ/kg DM) (12.7) among the treatments. Crude protein and ME were selected more with less plantain in the offered diet to equalise them in the ingested concentration.

5.2.3 Water intake

Drinking water intake was different among the treatments declining from 38.2 L/cow/day to 21.7 L/cow/day as plantain proportions increased from 0% to 60% in the diet, respectively. Feed water intake was averaged 109.9L/cow/ treatment and increased from PL0 to PL60 by about 25%. Edwards *et al.* (2015) estimated a feed water intake of 100 L/cow/day from summer diverse and simple pastures offered to mid lactating cows in their experiment. The total water intake increased as the plantain proportion increased in the diet. Earlier studies on water intake reported varying results for example Cardot *et al*. (2008), Castle and Thomas (1975), Jago *et al*. (2005) 83.6, 49.9, 53.7 litres drinking water for cows on total mixed rations, forage and hay and ryegrass grass respectively. In an indoor study and ryegrass pasture trial Morris *et al*. (2010), recorded total water intake of 110 and 104 litres respectively.

5.2.4 Milk yield and composition

A feature of the results was that milk production (fat, protein & yield) was unaffected by the proportions of plantain in the diet. The only tendency was for protein percent in the diet to increase from 4.42% in PL0 to 4.63% in PL60.

The lack of difference in milk production is surprising given that the feed intake for plantain included diets were higher than the pasture diet.. Similar WSC (2498.6g/day/cow) and CP (3873.0g/day/cow) ingested across the treatments could explain the similar milk production. The similar WSC and CP was due to different nutrient selection differentials decreasing with an increase in plantain proportions, equating the composition of the feed components. Metabolisable energy intake that was similar (203.4MJ/kg /cow/day) for the plantain included diets but higher than ryegrass-white clover (187.5MJ/kg /cow/day) was expected to increase milk production for the plantain containing diets, but it did not. This could be explained by Stockdale (2007) who outlined that in late lactating cows most of the energy is directed to lean tissues and lipids as opposed to milk production, which could also have an input on the similar milk production in this trial. In a related study, Al-marashdeh, Greenwood, Hodge, and Edwards (2015), concluded that the difference in DMI not translating to different milk yield and solids could be due to similar CP. Further, Edwards *et al*. (2015) reported similar DMI between simple and diverse pastures (15.3±2.1 *vs*. 16.2±1.4kg DM/cow/day respectively) that did not change the milk yield and composition. The body condition scores (4.2) and liveweight changes (0.17 kg/ha/day) that could have explained the similarities in milk production parameters were also not different amongst the treatments in this study. It could also be inferred that though there were no differences in BCS, the scores were higher for the cows in late lactation and therefore the suggestion by Stockdale (2007) that energy was dedicated to lean and fat tissues could be true. Minor differences in milk composition could be also be attributed to the low demand of energy for milk production in late lactating cows (Totty *et al*., 2013). Castle and Thomas (1975) found a positive significant relationship between water intake and milk production but despite the increased water intake with increase of plantain proportions, this did not affect the milk production.

5.3 Nitrogen partitioning

Urine N concentration and urine N output were 33% lower in PL60 than PL0 (0.30 vs. 0.45 g N/L respectively). The observed reduced N concentration in the urine was despite the fact that N intake was similar in all the treatments. These results suggest that although the N intake was the same amongst the treatments, the difference in urinary Nwas derived from 30% to 60% plantain in the diet with the difference in N concentration and N output between PL0 and PL30 being 13%. This result is comparable to Totty *et al*. (2013)'s study where N concentration declined from 0.57% in perennial ryegrass-white clover to 0.34% in diverse pastures with 36.1% chicory, 18.4% plantain and 0.2% lotus DM. Further Box *et al*. (2016) reported urine N concentrations of 5.4 g and 3.6 g N/L for pasture and 50% pasture: 50% plantain respectively. Woodward *et al*. (2013) reported 32% less urinary N in diverse pastures (29%) than pasture (43%), but in the studies it is not clear the contribution of plantain to N reduction as it was a mix with chicory and lotus. Judson and Edwards (2016) suggested that since the N reduction was similar in diverse and spatially separated plantain-ryegrass pastures, the effect could be due to plantain rather than the other herbs in the mix. The urinary N concentration difference beween PL0 and PL60 may have appeared numerically smaller in this study, but when this is factored in the estimation of N loading the difference was magnified with lower N loading for PL30 and PL60 than the other treatments.

Milk urea nitrogen (MUN) an indicator of urinary N excretion (Cosgrove, Taylor, Lowe, Foote, & Jonker, 2014) declined from 16.2 mmol/L in PL0 to 13.2 mmol/L in PL60. A similar pattern was observed by Totty *et al*. (2013) when they reported urea N concentration of 11.4 mmol/L and 9.5mmol/L from milk of cows grazing ryegrass and diverse pastures respectively. The reasons for urinary N decreasing with the increase of plantain proportions in the diet are not clear. Urine N excretion and concentration is linked to N intake (Edwards *et al*., 2015; Totty *et al*., 2013), however this was similar in this study (619.7 g/cow/day) to justify the urinary N differences. Although the N intake was not different, there was a trend for decreased N intake (from PL0 to PL60). It may be concluded that this small difference in N intake (eg 7.6% between PL0 and PL60) had an impact on reduced urinary N concentration. However the difference could be also be attributed to secondary plant compounds. According to Navarrete *et al*. (2016) and Tamura and Nishibe (2002) reduction of N in urine may be attributed to secondary bio-actives aucubins and acteosides that reduce NH_3 (Navarrete *et al*., 2016; Tamura & Nishibe, 2002). The levels of aucubin (4.63 mg/g DW) and acteoside (2.29 mg/g DW) in this study were similar (1.78-3.80 mg/g DW) and lower (23.6-35.4 mg/g DW) than those reported by Navarrete *et al*. (2016) in the first season of plantain growth in Palmerston North. In their invitro study aucubin (20 mg/g DW) reduced NH_3 concentration in the rumen possibly by inhibition of rumen microbial growth or rumen hyper-ammonia producing bacteria (Durmic & Blache, 2012). Acteoside at a higher level (36 mg/g DW) than in this study increased the gas production and acted as an energy source (Navarrete *et al*., 2016). In this study, WSC:CP ratio could not have influenced N utilisation as explained by Totty *et al*. (2013). The ratios of the concentration of intake of WSC:CP increased as the plantain proportion in the diet increased although all the values were lower (0.59, 0.64, 0.65,0.70 for PL0, PL15, PL30 & PL60 respectively) than >0.70 values needed to contribute to increased N utilisation (Edwards, Parsons, Rasmussen, & Bryant, 2007).

5.4 Purine derivatives and microbial N production

Purine derivatives (PDs) (allontoin and uric acid) were used to estimate supply of microbial protein which is essential for maintenance and production (Chen, Mejia, Kyle, & Ørskov, 1995; Singh *et al*., 2007). Microbial N supply (g N/day) in this trial was higher (395.3) than (217.3) that reported by Totty *et al*. (2013), although in each case the results among the treatments were similar. The differences could between the experiments could be that the latter authors used high sugar ryegrass and diverse pastures that included chicory, plantain and lotus which had a higher WSC:CP ratio (0.85) than the average (0.65) of the diets in this trial.

5.5 Animal behaviour

5.5.1 Time allocation and preference

In this study, the time allocation to grazing pasture declined as the proportions of plantain increased for both morning and afternoon grazing bouts. This could be that cattle select more with a larger strip of monoculture of a preferred species (Rutter, Cook, Young, & Champion, 2005). The latter authors discovered that cattle's preference increased from 37% in a small area (108 x12cm) to 60% in a larger strip area (108x 36cm) when grazing white clover and ryegrass. Considering the perennial ryegrass-white clover and pure plantain strips in this study, the cows allocated plantain more time than pasture in the morning grazing (47.3 vs. 43.5 minutes respectively). In the afternoon grazing bout, the reverse was true with time invested more in grazing pasture than plantain (62.1 vs. 34.9 minutes/cow respectively). This could have been because the plantain had been grazed out and depleted. Edwards *et al*. (2008) explained that animals graze species with more energy in the morning and grass in the afternoon so they can look out for predators as they ruminate. Although the energy and CP of the plantain and ryegrass/white clover and their intake were not different it could be still postulated that the most palatable with low NDF would be preferred first before the fibrous. Palatability of plantain is further explained by that it contains about 2% sorbitols and 60-70% sweetness of sucrose (Deaker *et al*., 1994; Grigore *et al*., 2015; Tamura & Nishibe, 2002).

In non-grazing activities, there was a tendency to reduce rumination with increasing plantain proportion in the diet although the results were not statistically significant. These results tie well with the fact that plantain is masticated fast, degrades and ferments better than pasture (Gregorini *et al*., 2013; Stewart, 1996). Idling activity numerically increased as plantain proportion rose in this study, a result that Cheng *et al*. (2015) also reported in heifers grazing chicory, plantain and ryegrass white clover.

5.5.2 Bite rate

The results of bite rate in this study (~45 bites/minute) were slightly lower than those reported by (Gregorini *et al*., 2013). The differences could reflect that in this study, the bite rate was estimated based on bites on pasture and plantain separately than on per treatment basis. The average bite rates of 46 in pasture versus 39 bites/minute/cow in plantain in the morning grazing and 49 *vs*. 44 bites/minute/cow respectively in the afternoon grazing all indicate that leafy herbage like plantain needs a larger bite mass (Anil & Forbes, 1988; Rattray *et al*., 2007). The higher and lower bite rates for grass and plantain respectively, was also reported by Gregorini *et al*. (2013) and Cheng *et al*. (2015). In another related study, Bryant, Miller, and Edward (2012) reported similar results (44 *vs*. 49 bites/minute for diverse and simple pastures respectively) and alluded this trend to variation of choice of species because of preference.

5.5.3 Urination behaviour

Urine volume data collected from 14 cows over 24 hour period per cow showed high variability. Urine volume per urination ranged from 1.33 to 3.85 litres (mean 2.32 litres), whilst 24 hour urine volume total per cow was 13-31 litres/cow/day. A similar high variability in volumes was reported by Ravera *et al*. (2015) (<1 -5 litres/urination event and 8.7-47.0 litres/24hrs per cow) and Edwards *et al*. (2015) (1.8-2.4 litres/urination event). The individual urine volume variability would make it difficult to estimate average N leakage in a urine patch without confirming with other measurements e.g. lysimeters data from the paddocks (Edwards *et al*., 2015). The urine volumes per urination in this study are in the range of those recorded by the latter authors under perennial ryegrass-white clover and diverse pastures.

The urination frequency in this study ranged from 7-16 times/24hr period per cow and on average was similar across treatments. The frequency is within the range of other studies e.g. Ravera *et al*.

(2015) (8-12 on kale and fodder beet); Edwards *et al*. (2015) (11.6-15 on ryegrass and diverse pastures) and Farrell (2015) (8.5-10.3 on kale and fodder beet forage). Similar figures were also confirmed by Clark *et al*. (2010) and Robichaud et al. (2011). In the study, the frequency of urination increased with plantain proportions, and this could be attributed to diuretic tendencies of plantain due to glycosides bio-actives and higher levels of mineral content (Navarrete *et al*., 2016; Tamura & Nishibe, 2002). After averaging, urine volumes per urination event did not respond differently to feed treatments, thus no evidence of diuretic effects of plantain as reported in other studies (Stewart, 1996). In sheep grazing plantain and ryegrass-white clover, O'Connell *et al*. (2016) produced evidence that plantain has a diuretic effect. Sheep on plantain produced higher urine volume by 1.7 L on the first day of the trial than those on ryegrass-white clover and 0.5 L more/day on 5 subsequent collection days.

After the calibrating the relationship of urine volumes and patch area, the estimated urine patch area were similar amongst the treatments averaging 0.34m^2. During data processing it was evident that the urine spread to cover a larger space on plantain but a smaller one on perennial ryegrass-white clover because of the varying density of the two pastures. The urine patch size in the study is similar to the ones estimated by Ravera *et al*. (2015) and Farrell (2015) who recorded 0.47 and 0.25m^2 and 0.22 and 0.25m^2 for kale and fodder beet respectively under wintering pregnant cows. Kale and fodder beet did not impede the movement of the urine and hence the slightly larger patches.

Urine N concentration and volume are key factors determining loading in a urine patch (Di & Cameron, 2007). Based on data from this study for urine volume, urine patch size (Table 4.13) and urine N concentration (Table 4.10) N loading (kg/ha) would be 316, 295, 281 and 190 for PL0, PL15, PL30 and PL60 respectively. There was a 40% decline from 316 to 190 kg N/ha in urine patch loading from 0% to 60% proportion of plantain in the diet indicating the key potential of plantain to reduce N loading. As urine loading is critical for determining N leaching, this is likely to contribute to reduce leaching even if urine frequency is marginally higher.

Chapter 6 Conclusion

6.1 Research contribution

This research succeeded in evaluating the effect of grazing spatially planted plantain and perennial ryegrass-white clover pastures on urinary N excretion, milk production and urinary and feeding behaviour of late lactating dairy cows grazing irrigated pastures in autumn. When plantain was included in proportions of 0, 15, 30 and 60% to ryegrass-white clover, the resultant diets had similar feed value to perennial ryegrass-white clover when offered as green leafy herbage to dairy cows. The protein concentration of the diets offered was similar amongst the treatments. The energy concentration though statistically different, numerically the margins were small. This therefore provided a fair basis for comparing the diets across treatments.

Despite the different proportions, plantain was grazed to the same height amongst the treatments containing it. Plantain has less cell wall, NDF and ADF than pasture; therefore the cows grazed it first in the morning before grazing pasture. The smaller proportions of plantain e.g. 15% and 30% were expected to be grazed lower than the 60%. However this did not happen and suggests that the cows moved on the pasture earlier on the 15% and 30% to maintain the similar grazing heights as the 60% plantain proportions. Whereas intake of CP was similar amongst the treatments, the DM intake was similar for the plantain included diets but higher than that for the ryegrass-white clover diet. Lower proportions of cell wall, cellulose and NDF for the plantain included diets may have made a higher intake difference than in the pasture based diet. It was expected that the DM intake would vary or show a trend of increasing as the percentage of plantain in the diets increased (from 15-60%) but this did not follow. This still needs to be further investigation.

More ME and DM intake for the plantain included diets than the pasture diet did not make any difference in milk production amongst the treatments as expected. This was difficult to comprehend leaving postulation that the difference in DM intake was not large enough to increase milk production in the plantain included diets. The other possible reason is that the extra ME and DM could have been directed to directed to lean tissues and lipids as opposed to milk production in late gestation as suggested by Stockdale (2007). These parameters could have also affected growth (weight gains), but however statistically no difference was observed except that there was a trend of weight increment with the increase in plantain inclusion from 15% to 60%. Final BCS were the same amongst the treatments, but higher than expected for the late lactation cows an indication that some of the energy in all treatments was used in the conditioning the animals. It may also be concluded that because of the similar microbial N production which is used for maintenance and production (although higher than that reported by Totty et al. (2013)), this was not sufficient to make a difference in milk production across the treatments. Dairy cows' milk production is also increased by energy production in the rumen. The glycoside acteoside in plantain has been found to increase energy and rumen gas production. In this study it appears that the acteoside's impact was not clear probably because their concentration was lower than that observed in other studies (Navarrete *et al.*, 2016) that had sampled plantain for more than one season.

Plantain has diuretic tendencies on livestock that are alluded to the presence of the glycosides and higher content of minerals (in this trial Na, Ca and S). In this trial the urine volume/ cow / treatment did not differ as expected even with expected diuretic effects of the minerals. The glycosides in the plantain were lower than those reported elsewhere probably due to the early stage of growth (approximately 3 months) of the plantain during the grazing trial. The method for collection of urination behaviour data (urine harness) is still being evaluated and therefore its accuracy still not well established. This could also have been a factor contributing to the same urination volume and frequency amongst the treatments. Despite the same urine volume, estimation of N loading using urine volume, urine patch size and urine N concentration, showed a difference amongst the treatments. The reduction of N loading by 40% between 0% and 60% plantain included diets in this research was an indication of better utilisation of N in the rumen and therefore less emission to the environment. This indicates that under favourable conditions for N leakage (rainy cold winters and free draining soils), it may be inferred that plantain inclusion reduces N impact on the environment.

Water intake was higher than that observed in other studies (Edwards *et al.* (2015) but with a trend. Drinking water intake decreased from 38.2 L/cow/day to 21.7 L/cow/day, but estimated feed water intake increased from 94.2 L/cow/day to 117.9 L/cow/day in PL0 to PL60 respectively. More water was consumed as the plantain increased in the diets. This made sense in that plantain is more succulent than pasture and therefore the more water consumed in the diets containing plantain, the less drinking water intake for those treatments. More water consumed though did not translate to more urine produced which was not clear and needs further follow up studies. Some studies (Castle & Thomas, 1975) indicated that there is a linear relationship between water intake and milk production, but in this case it was not so as the milk production was similar amongst the treatment. This indicates that milk production increment is a function of many factors like DM, protein and energy intake.

This study therefore confirmed that feeding plantain proportions between 30 and 60% results in reduction of in both N concentration and urine N excretion, whilst maintaining similar milk production at the level of that produced when cows are fed ryegrass-white clover diets. The next pasture management studies need to determine how diets with plantain proportions greater than 30% can be generated on farm at critical times of the year to reduce N leakage.

6.2 Potential for further research

6.2.1 Length of trial

The effects of plantain on milk production could better observed under a longer period of experimentation. This trial was done over 12 days and so had a gap that the evaluation of the time and treatment interaction in affecting milk production could not be done.

6.2.2 Plantain age

It has been found that the concentration of glycosides (especially aucubins and acteosides) in plantain increase with age (Navarrete *et al*., 2016; Tamura & Nishibe, 2002). Plantain in this trial was about 3 months old. A similar trial over 2 seasons may result in different results in terms of urine N concentration, N microbial yield and utilisation in the rumen considering that the glycosides and mineral concentration would have increased.

6.2.3 Urination calibration

It has been established in this and previous studies that urination frequencies of lactating dairy cows and volumes vary. It would be helpful in future to physically observe the urination duration and use this data with the urine harness one as a way of ground proofing. This would produce a comprehensive study with checks on the urine harnesses outputs.

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